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THE EXPERIMENTAL PRODUCTION OF GENERALIZED ARTERITIS AND PERIARTERITIS (PERIARTERITIS NODOSA)

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Chicago, Ill.

Among the various etiologies suggested for periarteritis nodosa, allergy has had a prominent place since Gruber[1] first suggested its possible causative role in certain cases which he observed. Recent clinical-pathologic studies by Rich[2, 3] have strengthened this view. Furthermore, Rich and Gregory[4] have shown that lesions characteristic of periarteritis nodosa can be produced in rabbits by the intravenous administration of large doses of sterile, normal, horse serum. Such lesions followed a single dose but were seen more regularly after multiple injections of the foreign serum. The significance of these observations is increased by the fact that lesions similar in many respects to those of rheumatic pancarditis were produced in the course of their experiments.[5, 6]

The clinical correlation of periarteritis nodosa with allergic phenomena of a different type has been made recently by Wilson and Alexander,[7] who found that bronchial asthma was present in fifty-four of 300 consecutive cases of this disease. Such a relationship is not apparent in all reports dealing with the same problem, however, and it has been stated that convincing evidence which points to any one responsible etiologic agent is lacking. Harris, Lynch, and O'Hare,[8] in a review of the recorded clinical cases of periarteritis nodosa, discussed those conditions which at one time or another have been considered as causative. In addition to an allergic mechanism these included such infections as may be produced by *Treponema pallidum*, streptococci, viruses, and protozoa. They stressed the point that the clinical course of periarteritis nodosa resembles that of a generalized infection with toxemia. The fact that a history of allergic disease was obtained in 15 per cent of their collected cases failed to impress them. More recently, Jones[9] has reviewed this subject and has considered either allergy, rheumatic fever, or infection of many types as a significant predisposing factor. In his opinion, however, none of these emerges as the dominant factor.

Thus, although periarteritis nodosa is a distinct entity on the basis of its characteristic gross and histologic features, it seems impossible, clinically, to attribute to it a single etiology. It becomes of primary importance then to seek the mechanism of development of this peculiar arteritis. Although this pathologic process may be affected by a variety of apparently unrelated cir-

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cumstances, there may yet be a single *pathogenesis*. Recent experimental data give support to this view. The studies of Rich and Gregory, as well as the ones which we shall describe, leave little doubt that the intravenous administration of large amounts of horse serum to rabbits can lead to a necrotizing and proliferative arteritis which, in both its acute and chronic stages, meets the essential microscopic criteria of periarteritis nodosa.

Using an entirely different approach, other investigators[10-12] have indicated recently that hypertension, experimentally induced, also may lead to a focal necrotizing arteritis and periarteritis. Smith, Zeek, and McGuire[13] have reviewed these and other similar reports. They have drawn attention to the fact that in all of these experiments, which include several species of animals (dogs, rats, and rabbits), either hypertension or renal disease were present. They record the occurrence of periarteritis nodosa in twenty-six of sixty-two rats and in four of eight dogs which they themselves made hypertensive. In their experiments hypertension was the result of a perinephritis induced by wrapping one kidney with silk. Seyle and Pentz[14] also produced lesions similar to those of periarteritis nodosa in rats made hypertensive by a somewhat different procedure. Following unilateral nephrectomy the animals were given large injections of desoxycorticosterone acetate; this was accompanied by an increased oral intake of sodium chloride. On the basis of experimental as well as clinical data, it would appear then that there is no single *etiology* for these focal arterial lesions.

The following experiments represent, first, a repetition of those of Rich and Gregory which, to the present time, apparently have not been repeated by other investigators. Second, considerable new data are presented which include serologic studies during the development of the various allergic lesions in the experimental animals. Third, certain aspects of our experiments have been designed to determine the underlying mechanisms responsible for the production of periarteritis nodosa.

MATERIAL AND METHODS

Sixteen white male albino rabbits, weighing from 2.7 to 3.9 kilograms and housed in individual wire-bottomed cages, were used. They were maintained on a commercial rabbit food* and water ad libitum, with the addition of approximately 150 Gm. of carrots two times per week. The horse serum used was sterile normal serum, without preservative, and was pooled from two sources.† A 10 per cent suspension of sterile powdered sulfadiazine was prepared in a 10 per cent solution of gum acacia in order to facilitate precise oral administration. This relatively stable suspension was administered by means of a stomach tube.

Rectal temperatures were determined at approximately 9 A.M. daily from the day before the first injection of horse serum through the twenty-sixth day of the experiment. The ears of the animals were carefully inspected daily from the fifth to the tenth day of the experiment and from the eighteenth to the twenty-fifth day for evidence of erythema and edema of the type originally described by Fleisher and Jones[15] as characterizing serum sickness in rabbits. Cutaneous reactions to 0.1 c.c. of horse serum or 0.1 c.c. of a suspension of sulfadiazine, injected intradermally, were observed on the eleventh and twenty-sixth days of the experiment. The sites of inoculation were inspected at one hour and at twenty-four hours

*Alpha Flakes.

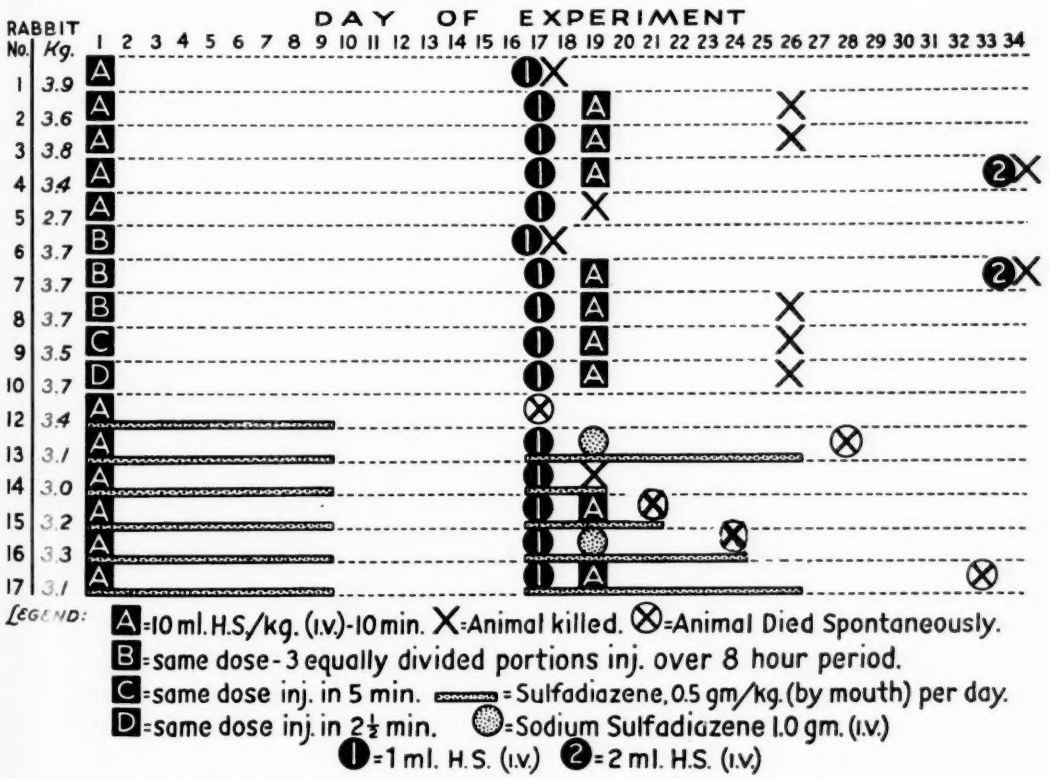
†Kindly donated by the Research Laboratories of Eli Lilly and Co., Indianapolis, Ind., and Lederle Laboratories, Inc., Pearl River, N. Y.

Antibody titers to horse serum were determined using the collodion particle agglutination technique of Cannon and Marshall.[16] Simultaneously, and using the same samples of serum, we attempted to demonstrate humoral antibodies to sulfadiazine by means of the serologic "inhibition" reaction. This was accomplished by making up a second series of dilutions of the antisera, using as the diluent a saturated solution of sulfadiazine in 0.86 per cent saline. This procedure allowed opportunity for the antiserum to combine with the sulfadiazine before the horse serum-coated particles were added. Tissues for histopathologic study were fixed in Zenker's solution, imbedded in celloidin, and stained with hematoxylin and eosin, Mallory's connective tissue stain, azan carmine, and Masson's trichrome stain (Goldner's modification).

EXPERIMENTAL PROCEDURES AND RESULTS

The animals were divided into two groups. Rabbits 1 to 10 received horse serum alone, while Rabbits 12 to 17 received sulfadiazine in addition to the horse serum (see Table I). It should be stressed that the type of experimental animal, various materials, dosages, and time intervals, as related to the injections of horse serum and sulfadiazine, followed, in so far as possible, those of Rich and Gregory.[4] For reasons which will be discussed later, the rate of injection of the initial dose of horse serum was varied as indicated in Table I.

TABLE I. TIME, AMOUNT, AND MODE OF ADMINISTRATION OF NORMAL HORSE SERUM AND SULFADIAZINE AND TIME OF DEATH


DAY OF EXPERIMENT
RABBIT No.
Kg.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
1 3.9
2 3.6
3 3.8
4 3.4
5 2.7
6 3.7
7 3.7
8 3.7
9 3.5
10 3.7
12 3.4
13 3.1
14 3.0
15 3.2
16 3.3
17 3.1
LEGEND:
A=10 ml. H.S./kg. (I.V.)-10 min. X=Animal killed. ⊗=Animal Died Spontaneously.
B=same dose-3 equally divided portions inj. over 8 hour period.
C=same dose inj. in 5 min. =Sulfadiazene, 0.5 gm/kg. (by mouth) per day.
D=same dose inj. in 2½ min. =Sodium Sulfadiazene 1.0 gm. (I.V.)
1=1 ml. H.S. (I.V.) 2=2 ml. H.S. (I.V.)

The rise in temperature[17] and the erythema and edema of the ears[15] characteristically exhibited by rabbits following injection of large doses of horse

TABLE II. DEGREE OF VARIOUS REACTIONS OBSERVED FOLLOWING ADMINISTRATION OF NORMAL HORSE SERUM (AND SULFADIAZINE) TO ALBINO RABBITS

RABBIT	TEMPERATURE RISE* (FOURTH TO NINTH DAY)	EAR REACTION† (FIFTH TO TENTH DAY)	ARTHUS REACTION‡ (ELEVENTH AND TWELFTH DAYS)	PRECIPITIN TITER§ (FIFTEENTH DAY)	TEMPERATURE RISE* (TWENTIETH TO TWENTY-FIFTH DAY)	EAR REACTION† TWENTIETH TO TWENTY-FIFTH DAY)	ARTHUS REACTION‡ TWENTY-SIXTH AND TWENTY-SEVENTH DAYS)	PRECIPITIN TITER§ TWENTY-SIXTH DAY)	VASCULAR LESIONS AT DEATH‖	DAY OF DEATH
1	1+	±	1+	480	—	—	—	—	±	17
2	3+	1+	3+	480	3+	0	—	960	3+	26
3	1+	3+	3+	480	4+	0	—	1,920	2+	26
4	2+	1+	2+	960	3+	±	3+	960	2+	34
5	2+	0	1+	960	—	—	—	—	3+	19
6	0	3+	3+	480	—	—	—	—	2+	17
7	1+	±	2+	960	3+	±	2+	1,920	1+	34
8	2+	0	2+	960	1+	0	—	960	3+	26
9	2+	1+	3+	480	3+	0	—	960	4+	26
10	1+	4+	2+	480	3+	±	—	480	1+	26
12	0	1+	1+	480	—	—	—	—	±	17**
13	1+	0	2+	240	1+	0	1+	480	±	28**
14	0	0	2+	480	—	—	—	—	±	19
15	2+	±	3+	960	—	—	—	—	1+	21**
16	1+	0	2+	480	4+¶	0	—	—	±	24**
17	0	1+	1+	240	0	0	1+	240	±	33**

0, Negative; —, not determined; ±, doubtful positive reaction.

*The average temperature during this period was compared with the average of a preceding five-day control period. An increase of less than 0.1° F., 0; 0.1 to 0.5° F., 1+; 0.5 to 1.0° F., 2+; 1.0 to 1.5° F., 3+; over 1.5° F., 4+.[17, 18]

†This evaluation was entirely subjective and was based on the intensity of the erythema and edema and its duration.[15]

‡Hyperemia with little or no edema, 1+; hyperemia with moderate to marked edema, 2+; marked hyperemia and edema with hemorrhage and central necrosis, 3+.

§Using the collodion particle agglutination technique (serial dilution of antibody).[16]

‖After the tissues had been thoroughly studied microscopically, grading of the vascular lesions in each animal was accomplished by means of subjective analysis based on the intensity and frequency of these lesions.

¶The cause of this temperature rise is questionable. During this period the animal appeared sick; at death there was marked obstructive nephropathy (sulfadiazine) and marked pulmonary edema.

**These animals died spontaneously.

serum, as well as the Arthus reaction and precipitin titers to horse serum, were carefully studied in an attempt to determine the extent of hypersensitivity exhibited by the animals at various intervals following the injections of horse serum. At the conclusion of the study the quantitative character of these ante-mortem reactions was compared with the intensity and frequency of the vascular lesions observed. In order to facilitate this comparison, the results have been condensed and tabulated in Table II. Of these data several points are particularly noteworthy. It is quite apparent that the animals receiving both sulfadiazine and horse serum, as compared with those which received the same doses of horse serum alone, exhibited a markedly lowered precipitin titer, temperature response, etc. Not only were these allergic and serologic reactions to horse serum consistently less, as judged by the observations made before death, but the frequency and intensity of the vascular lesions were also much less in the group that received horse serum and sulfadiazine. It appears thus that there is a gross correlation between antibody response and the extent and degree of arteritis following the injection of large

amounts of horse serum. When either of the groups is studied alone, however, it becomes apparent that there is no consistent correlation between ear reactions, Arthus reactions, or precipitin titers and the type or extent of vascular response apparent at autopsy. The temperature rise which occurred following the first injection of horse serum, in the animals receiving horse serum alone, did tend to parallel the degree and extent of arteritis observed at death. We cannot explain the marked diminution in ear reactions following the second large dose of horse serum, unless the reactions were all of the "immediate" type[18] and hence so transient as to escape our daily observations.

In addition to the data recorded in Table II, skin tests performed with a 10 per cent suspension of sulfadiazine in saline on the eleventh day of the experiment were negative in Rabbits 12, 15, and 17 but were doubtfully positive in Rabbits 13, 14, and 16. In these latter animals a 5 by 5 mm. area of edema and slight erythema was observed at twenty-four hours, whereas similar intracutaneous injections in four animals which had received no sulfadiazine previously produced no reaction. Attempts to demonstrate circulating antibodies to sulfadiazine by means of the serologic "inhibition" reaction, utilizing serum obtained on the fifteenth day of the experiment, were completely unsuccessful. Under these conditions, inhibition of the precipitin reaction might have been expected, if antibodies had been formed to a sulfadiazine-horse serum complex.

Yet another ante-mortem observation of considerable interest was the appearance of a maculopapular erythematous eruption over the abdomens of four rabbits following the second large injection of horse serum (nineteenth day). The hair had been clipped nine days earlier in preparation for skin testing; at the time of the erythematous response the Arthus reaction had subsided completely. This eruption appeared in Rabbits 2 to 4 from the twenty-first to the twenty-third day and was seen on the twenty-second and twenty-third days in Rabbit 9, following which it faded rapidly. The erythema was marked, and there was no indication of trauma nor other extraneous stimulus as a cause. Why the ears of the rabbits did not react at the same time is difficult to explain, but the time of appearance and disappearance of this rash corresponds so well with that of "accelerated serum sickness" in rabbits,[18] which might be expected following this second injection of horse serum, that it is difficult for us to dismiss the lesions as coincidental. If the aural erythema and edema described by Fleisher and Jones[15] is analogous to human serum sickness, one would expect that it would not necessarily be localized to the ear of the rabbit. A biopsy of this dermal lesion from Rabbit 2 was obtained on the twenty-third day of the experiment. Microscopic examination revealed a superficial dermatitis characterized by moderate diffuse infiltration of leucocytes, edema, and a pronounced hyperemia of the small veins. Most of the infiltrating cells were mononuclear, although polymorphonuclear leucocytes were present. In the subdermis the collagenic fibers were swollen and vacuolated and occasional areas exhibited fibrinoid necrosis. The inflammatory process diminished in intensity in the subcutaneous region where it was localized about small blood vessels, chiefly veins (Fig. 1).

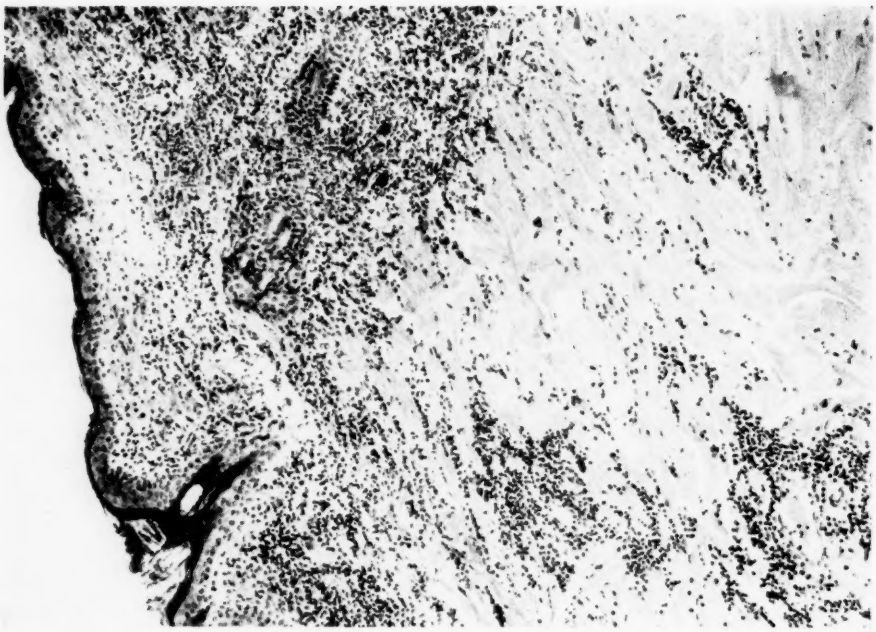


Fig. 1.—Rabbit 2, twenty-third day. Biopsy of erythematous eruption of skin showing infiltration by lymphocytes, focal areas of necrosis, edema of the superficial dermis, and hyperemia (×125).

Fig. 2.—Rabbit 9, twenty-sixth day. Section of a coronary artery showing marked periarteritis and necrotizing arteritis with almost complete occlusion of the lumen by infiltrating cells, most of which are lymphocytes and macrophages (×155).

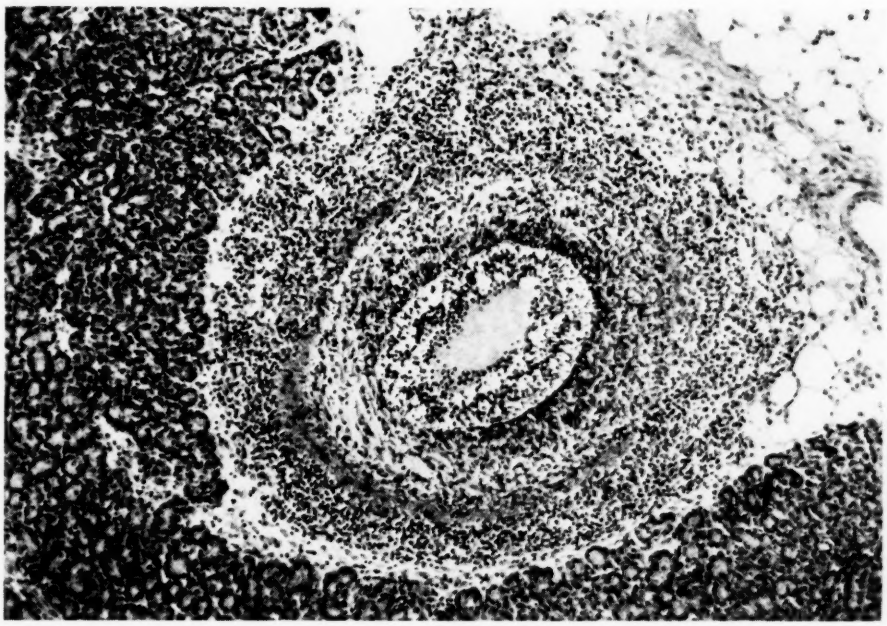


Fig. 3.—Rabbit 9, twenty-sixth day. Section of the pancreatic artery showing marked fibrinoid necrosis of the media accompanied by a marked arteritis and periarteritis (×130).

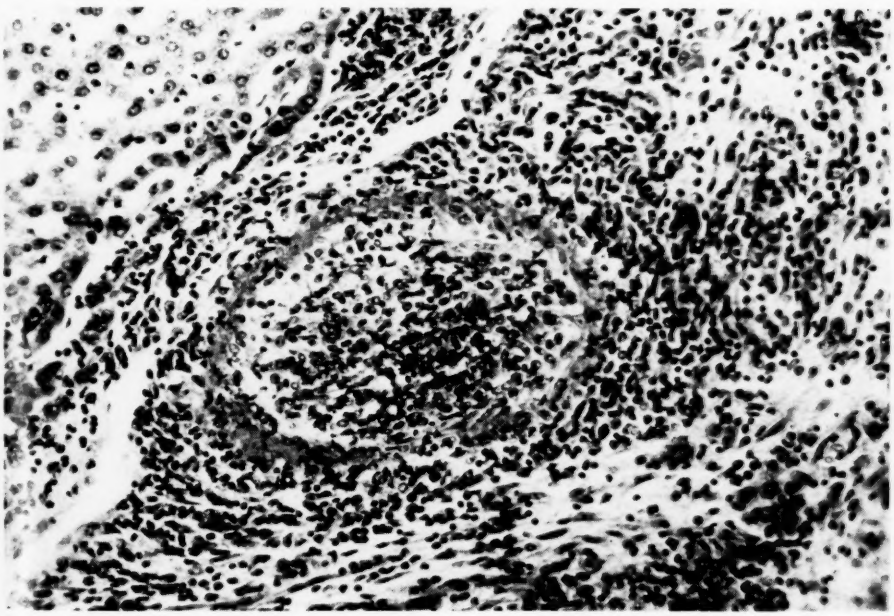


Fig. 4.—Rabbit 9, twenty-sixth day. Section of an hepatic artery with a similar arteritis and periarteritis. The lumen has been reduced to a small slit (×285).

POST-MORTEM FINDINGS

Gross Findings.—Moderate to marked enlargement of the spleen was the most constant gross finding. In addition, four of the six animals (Rabbits 12 to 15) receiving sulfadiazine showed moderate to marked enlargement of the kidneys. Cut surfaces exhibited a yellow-white granular precipitate arranged in strands paralleling the long axis of the pyramids. In four animals (Rabbits 3, 12, 14, and 16) the thymus was markedly atrophic. Two animals of the group (Rabbits 13 and 16) receiving sulfadiazine presented marked pulmonary edema and hyperemia. These animals were the two which had received sodium sulfadiazine intravenously on the nineteenth day; they died unexpectedly on the twenty-eighth and twenty-fourth days, respectively. Slight parasitic infection of the liver or mesentery was observed in four animals (Rabbits 1, 8, 9 and 14). In one case the organisms were identified as encysted tapeworms and in another as coccidia. None of the arterial lesions described below was grossly apparent, and there were no obvious manifestations such as thrombosis, infarction, aneurysm formation, or hemorrhage which might have suggested their presence.

Microscopic Findings.—

Heart and Blood Vessels: Vascular inflammatory changes similar to those described by Rich and Gregory were the most frequent lesions encountered. Only one animal (Rabbit 9), however, showed a marked *generalized* necrotizing arteritis (Figs. 2 to 4). These lesions were seen in the small and medium sized arteries of the heart, liver, pancreas, spleen, thymus, and lymph nodes. Some of the larger arteries showed focal necrosis and acute inflammation of their walls (Fig. 5). Acute and subacute periarteritis, characterized by polymorphonuclear and/or mononuclear leucocytic infiltration of the adventitia was seen in eleven of the sixteen animals (Figs. 6 and 7). Proliferative lesions of the endothelium and chronic inflammatory changes of the media and adventitia were commonly observed (Rabbits 2, 7, 9, 10, and 14). In these, the cellular infiltrate was composed of lymphocytes and macrophages predominantly, and there was usually an associated medial and periarterial fibrosis of varying degree. The earliest changes appeared to be a "loosening" of the adventitial connective tissue with edema and a delicate infiltration of monocytic cells. Accompanying this, a similar change in the medial layer was often observed. This apparently preceded the fibrinoid necrosis and actual disruption of the media. Accompanying the necrosis there was proliferation and intercellular edema of the endothelium. At this stage cellular infiltration was often very marked, and there was an almost complete obliteration of the vascular lumen (Figs. 2 and 4). Fibrosis of the media and adventitia appeared to be a sequel to this. These various stages of arteritis were most frequently seen in the heart, although they were observed in other organs. This distribution is similar to that reported by Vaubel[19] and by Miura[20] who produced arterial lesions in rabbits by repeated injections of foreign serum. Focal periphlebitis of a slight degree was observed in several instances.

Lesions with some of the characteristics of Aschoff bodies were encountered occasionally (Fig. 8). Unfortunately, our observations did not include

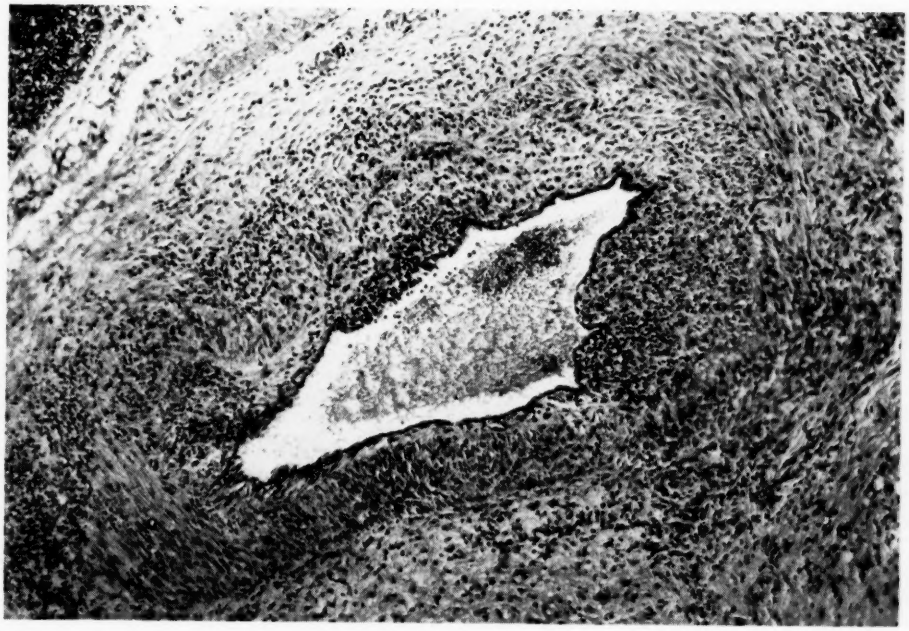


Fig. 5.—Rabbit 9, twenty-sixth day. Focal necrosis, arteritis, and periarteritis of a large artery near the thymus (×120).

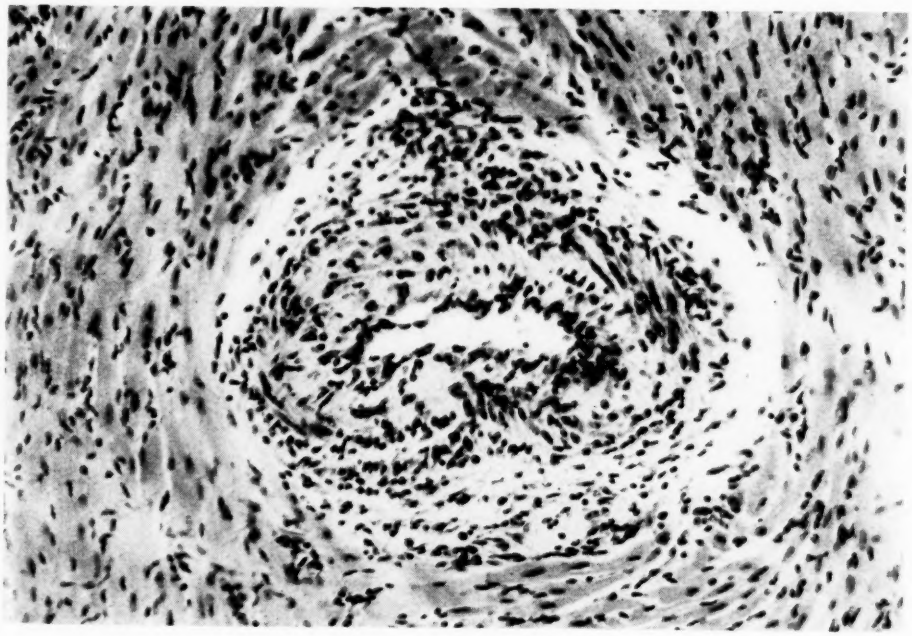


Fig. 6.—Rabbit 4, thirty-fourth day. A small branch of a coronary artery showing periarteritis. Although most of the inflammatory cells are lymphocytes, polymorphonuclear cells are present (×235).

Fig. 7.—Rabbit 9, twenty-sixth day. Section of myocardium showing round cell infiltration around a small artery (×265).

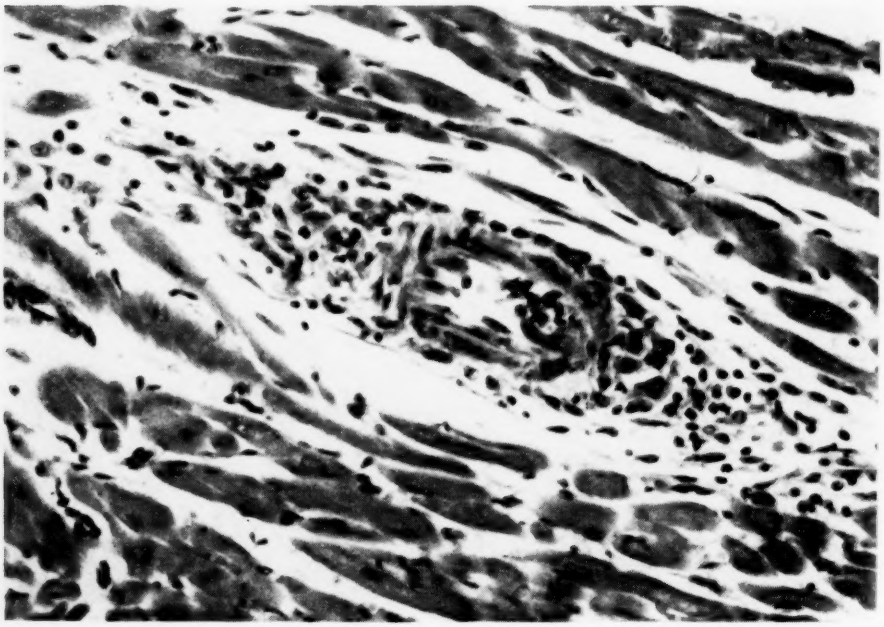


Fig. 8.—Rabbit 8, twenty-sixth day. This perivascular lesion in the myocardium resembles an Aschoff body (×450).

Fig. 9.—Rabbit 14, nineteenth day. Section of the thoracic aorta showing cystic necrosis of the media. This lesion was present throughout the entire circumference at this level (×275).

Fig. 10.—Rabbit 15, twenty-first day. This focal area of cystic medial necrosis lies in the thoracic aorta. Note the fibrinoid necrosis and infiltration of large mononuclear cells which resemble Anitschkow myocytes (×250).

adequate histologic study of the heart valves. Twelve of the sixteen animals exhibited a slight to moderate focal interstitial myocarditis (Fig. 7). These lesions consisted of dense accumulations of inflammatory cells, predominantly lymphocytes and plasma cells. In one animal (Rabbit 14) there was marked medial necrosis of the aorta which resembles that described by Erdheim[21] and by Moritz.[22] This lesion was seen in the proximal descending aorta and affected the midportion of the media. It involved the entire circumference, and many small cystic areas were seen (Fig. 9). There was no significant inflammatory reaction. The aorta of Rabbit 15 showed a lesser degree of medial necrosis; here the process was focal and appeared more acute. There was fibrinoid necrosis of the collagenic and elastic tissue of the media accompanied by an infiltration of large cells which resembled Anitschkow myocytes (Fig. 10). Sections of aorta were available from only eight of the sixteen animals.

In addition to the vascular lesions, the following pathologic changes warrant special comment.

Kidneys: Five of the six animals which received sulfadiazine showed moderate to marked obstructive nephropathy from sulfadiazine (Fig. 11). Obstruction of the collecting tubules by crystals of the drug had produced marked dilatation of the proximal portion of the nephrons. In addition, the epithelium of the collecting tubules adjacent to the impacted crystals showed rather marked degeneration and necrosis in some areas; in other areas there were foci of marked epithelial hyperplasia. Another striking change observed in the kidneys was a moderate to very marked proliferation of the juxta-glomerular apparatus. This occurred in approximately one third of the animals (Fig. 12). The significance of this finding will be discussed later. Focal acute glomerulitis with occasional synechiae and a rare instance of "crescent" formation were observed in four animals (Rabbits 1, 3, 8, and 9). In two of these animals (Rabbits 1 and 8) there was, within tubules considerable protein precipitate and a few erythrocytes.

Spleen: In addition to a fairly constant follicular hyperplasia, there was proliferation of loose connective tissue about many of the Malpighian arteries appearing as a relatively cell-poor, net-like central area within the corpuscle. The splenic sinuses of many of the animals contained an increased proportion of polymorphonuclear leucocytes, and in some there was considerable blue-staining granular material resembling nuclear debris, much of which was within macrophages. This latter change was seen most frequently in animals which died shortly (one or two days) after injection of horse serum.

Liver: Although the majority of the animals showed a slight to moderate increase in fibrous connective tissue in the portal triads, accompanied by focal collections of inflammatory cells, the significance of these changes was obscured by the parasitic infection present within the group. Occasional areas of recent or healing focal necrosis, not in association with portal triads, were observed in three animals (Rabbits 1, 6, and 9). Hartley and Lushbaugh[23] have reported focal hepatic necrosis in hypersensitive rabbits following injection of specific antigen.

Fig. 11.—Rabbit 14, nineteenth day. Section through the medulla of the kidney showing marked distention of the collecting tubules by sulfadiazine crystals accompanied by focal necrosis and acute interstitial nephritis (×125).

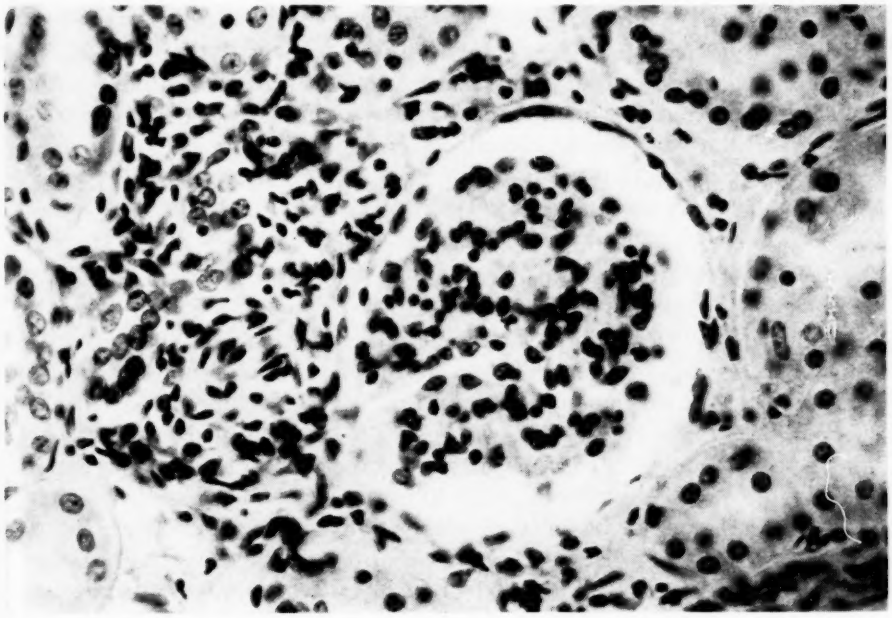


Fig. 12.—Rabbit 2, twenty-sixth day. Section of the cortex of the kidney showing marked proliferation of the juxta-glomerular apparatus (×625).

Serous Membranes: Focal fibrous thickening of the peritoneum associated with slight cellular infiltration (lymphocytes and polymorphonuclears) were seen in five animals.

Bone Marrow: Although leucocyte or erythrocyte counts were not determined in this series, a marked hypoplasia of the femoral marrow was observed in one animal (Rabbit 16). Death in this animal apparently resulted from an overwhelming pulmonary infection. In spite of the large numbers of bacteria which were present in the protein-rich edema fluid of the lung, no polymorphonuclear leucocytes were evident. This appearance is quite typical of that observed in human beings dying of agranulocytosis (malignant granulocytopenia). No polymorphonuclear leucocytes were seen in this very hypoplastic femoral marrow. Hypoplasia of the marrow was encountered in two other animals of the group which had received sulfadiazine and horse serum. Additional experiments to determine more precisely the effect of such treatment upon circulating leucocytes and the bone marrow have been performed and will be reported later.

DISCUSSION

We have demonstrated that large doses of horse serum given intravenously, alone or in combination with orally administered sulfadiazine, will produce arterial lesions in rabbits which appear identical with those of periarteritis nodosa. This confirms the observations of Rich and Gregory.[4] This fact in itself does not, however, answer the following questions: Is the inflammatory response due to a focal "hypersensitive" reaction? If so, why does it localize in small and medium-sized arteries rather than in those organs which are thought to form antibody and which presumably contain antibody in greater concentration (spleen, lymph nodes, liver, bone marrow)? If, on the other hand, these arterial lesions are due to the intravascular action of a toxic antigen-antibody complex, why are not the most marked lesions produced where the circulation is slowest and the vessels most delicate (veins and capillaries)?

The fact that hypersensitivity is associated with a wide variety of inflammatory lesions has been well established experimentally. The most familiar of these are: (1) the Arthus phenomenon, which can vary from a focus of slight erythema to a massive hemorrhagic and necrotizing lesion, and (2) the Auer phenomenon, in which localization of the allergic reaction can be brought about independently of any local instillation of antigen or antibody and in which the lesion may be principally proliferative in certain stages. In experimental animals, glomerulonephritis, a proliferative inflammatory response, can be produced by allergic reaction using nephrotoxin (antibody specific for renal tissue). From a clinical aspect, the direct relationship of serum sickness, asthma, contact dermatitis to the hypersensitive state has long been evident. With reference to the specific problem of periarteritis nodosa, however, one might ask why this disease, if it has an allergic basis, does not occur more often following such routine clinical procedures as passive sensitization with antitetanic horse serum. We are inclined to agree with Rich and Gregory that it is probably a matter of dosage. In this laboratory we had not previously observed vascular phenomena of the sort described here, although in

numerous instances rabbits had been injected repeatedly with various antigens including horse serum; never before, however, had such large doses of antigen been given. The type of antigen employed is unquestionably important also. Horse serum, with its relatively large molecular components, has been shown to persist in the circulation of a normal rabbit for fifteen days or longer.[24, 25] Its disappearance at this time coincides with the development of active immunity.

Ante-mortem observations made during the course of our experiments demonstrate that circulating antibodies to the horse serum were present while the vascular lesions were developing. A state of hypersensitivity was demonstrated also by the positive Arthus and ear reactions, and the elevations of temperature observed. The lack of quantitative correlation between these reactions is probably dependent upon the fact that horse serum contains multiple antigens. Thus, it is possible that the various manifestations may have been related to different antigen-antibody reactions. For example, one component of the serum might have been principally responsible for the temperature response and another for the ear reaction. In this regard, it is of interest that Jones and Fleisher[26] found that the pseudoglobulin portion of horse serum contained the substance responsible for "serum sickness" in rabbits. It is pertinent also that various procedures used commercially in the treatment of immune serums effect a marked reduction in the "serum sickness factor" with but little apparent effect on the antibody fraction. Further experiments are indicated to determine whether single purified proteins in large doses can produce an arteritis and periarteritis. Under the conditions of such an experiment, quantitative correlation among the various immune and allergic reactions might well be apparent.

Concerning the vascular localization of these inflammatory lesions, we originally considered that there might have been an initial injury to small arteries and arterioles resulting from the sudden infusion of a large amount of serum (an amount equal to approximately one-seventh the total blood volume). A necrotizing antigen-antibody reaction localizing at such a site of injury would be somewhat comparable to that illustrated by the Auer phenomenon.[27] Accordingly, the rate of the initial injection of horse serum was widely varied in our rabbits in order to produce differing degrees of vascular "overloading." It was found that the rate of infusion (whether two and one-half minutes or over a period of eight hours) bore no relation to the severity of the arterial lesions that resulted.

Since a characteristic response to anaphylaxis in rabbits is spasm of small arteries and arterioles,[28-30] it is possible that the combination of circulating antigen with antibody, as it is slowly formed and liberated, might lead to a persistent diffuse or focal arterial and arteriolar spasm. Certainly the aural changes described by Fleisher and Jones,[15] as characteristic of serum sickness in rabbits, indicate a marked vascular response. The fact that Rich and Gregory lost three of fourteen animals from anaphylactic shock at the time of the second and third injections of horse serum demonstrates that this method of sensitization can prepare rabbits for this type of allergic reaction.

In our series of sixteen rabbits, none died of anaphylactic shock, although three exhibited moderate dyspnea after the second injection.

The observations[10-14] that vascular lesions, similar in many respects to periarteritis nodosa, can follow experimentally induced renal hypertension in rats, rabbits, and dogs contributes additional evidence that vascular spasm may provide a *locus minoris resistentiae* in effecting the localization of inflammatory reaction to small arteries. Wilson and Byron[11] have suggested that marked arteriolar constriction and the accompanying hypertension act together to produce arterial necrosis in hypertensive animals. Others stress the fact that in the majority of instances in which necrotizing and proliferative arteritis complicates experimentally induced hypertension, there has been an associated infectious process.[12, 13, 31] It is altogether possible that such infectious lesions provide the stimulus for hypersensitivity which, in conjunction with arteriolar spasm, effects an allergic arteritis. It may be that the very marked hyperplasia of the juxta-glomerular apparatus which was observed in a number of our animals was evidence of hypertension. Hyperplasia of the juxta-glomerular apparatus, similar to that observed here, is frequently seen in human cases of hypertension and is an almost constant feature of some types of experimental hypertension. It has been suggested that in such instances this myoneural apparatus causes a persistent spasm of the afferent glomerular arterioles and produces renal ischemia which is in turn responsible for hypertension. We are inclined to believe that arterial injury from prolonged constriction or spasm may provide a localizing mechanism which contributes to the development of periarteritis nodosa. The frequent occurrence of hypertension in periarteritis nodosa is well known. Of 177 cases of human periarteritis nodosa reviewed by Logue and Mullins,[32] 53 per cent exhibited hypertension. Of the eleven cases which they themselves carefully studied, they state, "Hypertension was present in every patient at some time during the course of the disease." Studies are in progress to determine whether or not experimentally induced periarteritis nodosa is accompanied by hypertension and, if so, whether the hypertension exerts some causal influence or is simply an effect.

The mechanism previously described would seem to explain the marked similarity between those vascular lesions resulting from experimental hypertension and those observed in rabbits following large doses of a foreign serum. The medial necrosis observed in the aortas of Rabbits 9 and 15 might be explained on a similar basis except that here the arterioles of the vasa vasorum were involved.

Our observations differ in several respects from those of Rich and Gregory. They found that animals which received both horse serum and sulfadiazine exhibited widespread necrotizing and inflammatory arterial lesions of essentially the same type and degree as did those animals which received only horse serum. In our series this was not the case (Table II). However, Rich and Gregory found that the daily administration of 0.5 Gm. per kilogram of sulfadiazine was not toxic to rabbits; whereas five of the six rabbits in our series which received this dosage died before the end of the experiment, and at autopsy they presented a moderate to marked obstructive nephropathy

due to the presence of sulfadiazine crystals in the collecting tubules. It is possible that this toxic reaction may have so injured the affected animals that their ability to produce antibodies was considerably depressed, leading in turn to diminished allergic response and the less severe arterial lesions which occurred. The vascular lesions observed by us were qualitatively the same as those described by Rich and Gregory, but even in the animals receiving horse serum alone they were not as numerous nor were they so generalized. Correspondingly, the febrile reactions and erythema of the ears which we observed appear to have been somewhat milder. Careful histopathologic examination did not reveal the acute diffuse glomerulonephritis which Rich and Gregory observed in ten of their fourteen animals. In four of our rabbits there was microscopic evidence of focal glomerulitis, with an occasional synechia and a rare instance of crescent formation. In two of these animals erythrocytes and hyaline casts were seen in the tubules. However, the focal nature and mildness of these lesions were remote from the typical picture of glomerulonephritis in human beings.

SUMMARY AND CONCLUSIONS

In an attempt to produce experimental periarteritis nodosa, a group of sixteen white male adult rabbits was injected intravenously with large doses of sterile normal horse serum. The rate at which this first injection was given was intentionally varied in an attempt to produce differing degrees of vascular "overloading." At certain intervals following this first injection, intravenous horse serum was again administered to most of the animals. During the experimental period six of the rabbits received two nine day "courses" of sulfadiazine by mouth; however, five of them failed to survive the second peroid of treatment. Rectal temperatures were determined daily for each animal from the first to the twenty-sixth day. At appropriate intervals their ears were examined for erythema and edema of the type described by Fleisher and Jones. Cutaneous hypersensitivity to horse serum and sulfadiazine was determined, and precipitin antibodies to horse serum (and sulfadiazine) were titrated. At varying times, from the seventeenth to the thirty-fourth day, the rabbits were autopsied and representative tissues taken for microscopic study.

1. The majority of the rabbits exhibited definite but varying degrees of "serum sickness," cutaneous hypersensitivity, and precipitin formation following the administration of horse serum. In general, there was poor quantitative correlation among the results of these ante-mortem observations and the degree and extent of arteritis observed post mortem. The relationship of the multiple antigenic proetins in horse serum to this lack of correlation is discussed.

2. Arterial lesions, varying from a generalized acute necrotizing arteritis and periarteritis to a less spectacular and more focal subacute and proliferative arteritis and periarteritis, were observed in the majority of the animals. These latter lesions especially were seen most frequently in the heart. On the whole, the vascular lesions observed were qualitatively similar to those described by Rich and Gregory but quantitatively less marked and less generalized.

3. As a rule, the most marked arterial lesions were observed in animals which had shown the greatest elevations in temperature. The most marked arteritis occurred in rabbits killed on the nineteenth and twenty-sixth days. There was no correlation between the rate at which the initial injection of horse serum was given and the intensity or frequency of the resulting arterial lesions.

4. As a group, the animals receiving sulfadiazine in addition to the horse serum produced less precipitin antibody and manifested a lower degree of temperature response and cutaneous sensitivity than did those animals which received horse serum alone. They also exhibited much less arteritis histologically. This may have been related to the toxic obstructive nephropathy which occurred in five of the six animals receiving sulfadiazine.

5. Attempts to demonstrate cutaneous hypersensitivity or circulating antibodies to sulfadiazine resulted in questionable or negative reactions.

6. A diffuse erythematous eruption in the skin of the abdomens of four rabbits was observed following the second large dose of horse serum. Histopathologic changes, as well as the time of their occurrence, suggest that they were cutaneous manifestations of "accelerated serum sickness."

7. Lesions resembling Aschoff bodies were observed occasionally in the myocardium, and subacute focal interstitial myocarditis was common. The aortas of two rabbits showed a cystic medial necrosis.

8. In addition to the obstructive nephropathy, there was a marked hyperplasia of the juxta-glomerular apparatus in the kidneys of approximately one third of the animals. The possible significance of this lesion is discussed. A slight focal glomerulitis was observed in four animals.

9. Toxic or allergic damage to the bone marrow is suggested by the fact that one animal developed agranulocytosis and two others exhibited rather marked hypoplasia of the bone marrow.

10. It is suggested that in the arteritis and periarteritis which follows large doses of horse serum, prolonged spasms of the small and medium-sized arteries may provide the *locus minoris resistentiae* necessary to localize this allergic inflammatory reaction. The possible pathogenic relationship of such allergic arteritis and periarteritis to similar lesions observed in experimental hypertension is discussed.

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PARACOLON BACILLI AND THEIR RELATION TO URINARY TRACT INFECTIONS

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PARACOLON bacilli, commonly regarded as aberrant or variant forms of the typical coliform bacilli, are generally described simply as late-lactose-fermenting strains of Escherichia and Aerobacter. This is, indeed, their chief common characteristic, and although they may differ from typical coliform bacilli in other cultural reactions, they are most noted for their failure to ferment lactose promptly with the production of marked acid and gas as do typical strains of Escherichia and Aerobacter.

This group of gram-negative bacilli has recently attracted considerable attention in certain fields of bacteriologic investigation. They have been studied by the sanitary bacteriologists to whom they are of interest as a possible index of the fecal pollution of water whose presence is not detected by the accepted methods of water analysis based on the prompt fermentation of lactose with acid and gas.[1-3] They have also been the subject of extensive investigation by the public health laboratories engaged in the study of enteric infections and outbreaks of acute gastroenteritis.[3-5] Here this group of gram-negative bacilli are of interest not so much as possible etiologic agents in intestinal disease, although the possibility of their significance in gastroenteritis has frequently been suggested,[1, 3, 4, 6, 7] but rather because their close cultural resemblance to the true enteric pathogens (Eberthella, Salmonella, and Shigella) often makes their differentiation a problem.[3-5, 7-12] Frequently they are falsely identified as one of the pathogenic, intestinal gram-negative bacilli, thus leading to misinterpretation of the significance of their presence in stool specimens from patients and carriers. This error these public health laboratories are striving to eliminate through a more thorough study and knowledge of the characteristics of these organisms. Last, certain of the paracolon bacilli have been used for genetic studies as organisms which readily lend themselves to the investigation of bacterial variation.[10, 11, 13]

With the exception of these fields, very little attention has been directed to the paracolon bacilli. Their occurrence in clinical material other than stool specimens has not been reported, with the exception of a few instances of their isolation from urine cultures, as will be reviewed later in this paper. Accurate information concerning them is not widely disseminated, and complete descriptions are to be found only in a few reports in the literature[1-4, 8] Textbooks of medical bacteriology contain at best only brief references to these organisms, and these descriptions are too inadequate to permit recognition of the various types of paracolon bacilli on this basis. The difficulty in identifying many of

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the paracolon bacilli, and the fact that the knowledge of and interest in these organisms appears to be confined to a few fields, suggests the possibility that their identity as such is not recognized by many and therefore the frequency of their occurrence is unknown. In any case, it is apparent that the relation of paracolon bacilli to infections other than those of the intestinal tract deserves investigation.

The isolation of a considerable number of paracolon bacilli from urine specimens has prompted the investigation of these organisms along several lines. We have been interested, first, in the cultural characteristics of paracolon bacilli and methods for their identification; second, in their significance in the urinary tract; and, third, in their relation to typical coliform bacilli from the same source. This report will concern itself with these three phases of the problem.

DESCRIPTION OF PARACOLON BACILLI

Excellent descriptions of the cultural characteristics of the various types of paracolon bacilli are to be found in the recent literature. Perhaps the best are those of Stuart and his co-workers. In 1940, Stuart, Mickle, and Borman[3] described the variations in lactose fermentation. In 1943, Stuart, Wheeler, Rustigian, and Zimmerman[4] elaborated the earlier description and reported the characteristics of paracolon bacilli as of the Escherichia, Aerobacter, and intermediate groups. In 1944, Borman, Stuart, and Wheeler[8] suggested the establishment of a new genus, the Paracolobactrum, to include these organisms and proposed the following three species: *Paracolobactrum aerogenoides,* for those related to the Aerobacter genus; *Paracolobactrum intermedium,* for those resembling the intermediate coliform bacilli; and *Paracolobactrum coliforme,* for those related to the typical Escherichia. Additional information concerning these organisms may be found in the publications of Parr and associates.[1, 2, 9-11]

TABLE I. CULTURAL CHARACTERISTICS OF TYPICAL COLIFORM BACILLI

GENUS	MOTILITY	LACTOSE	DEXTROSE	SUCROSE	MANNITOL	INDOL	MALONATE OR CITRATE
Escherichia	+/-	AG 24 hr.	AG	AG/-	AG	+	-
Aerobacter	-	AG 24 hr.	AG	AG	AG	-	+
Intermediates	+/-	AG 24 hr.	AG	AG/-	AG	+/-	+/-

AG, Acid and gas.
+/-, Positive or negative.

It is not the purpose of this report to review in detail all the accumulated knowledge concerning the paracolon bacilli but rather to give as simply as possible an adequate description of these organisms, particularly as they occur in urine cultures. It is difficult to give a good, concise description of the paracolon bacilli because of the multiplicity of cultural types which may be encountered. According to various authors, they may arise from any of the coliform bacilli, that is, from Escherichia, Aerobacter, or the intermediate group.[1, 3-5, 8, 9] The cultural characteristics of these typical coliform bacilli are given in Table I.

As a result of this proposed difference in origin, the paracolon bacilli will differ in their cultural reactions as do the groups from which they arise. This alone produces a number of cultural types. But the paracolon bacilli are characterized by their variation from these typical coliform bacilli. In the first place, they vary considerably in their fermentation of lactose. Typical coliform bacilli, that is, Escherichia, Aerobacter, and the intermediate types, ferment lactose promptly, within twenty-four hours, with the production of acid and marked gas (20 per cent or more). The aberrant coliform bacilli, as the paracolon bacilli are frequently called, may have any of the following atypical reactions in lactose[1-4]:

1. Ferment lactose slowly (forty-eight hours and over) with acid and gas.
2. Ferment lactose slowly with acid and slight gas. Gas production may vary from a bubble to 10 per cent.
3. Ferment lactose slowly with acid and no gas.
4. Ferment lactose slowly and atypically at 37° C. but give a typical acid-plus gas reaction at 20° C. These organisms have been isolated only from water, milk, and soil and not from human material.[3]
5. Fail completely to ferment lactose.

It is these differences in lactose fermentation which are generally considered the chief characteristic of the paracolon bacilli. However, it must be understood that these organisms may differ in a number of characteristics other than lactose fermentation, as has been repeatedly pointed out by investigators.[1, 3, 4, 9, 11, 13] They may fail to ferment or may ferment atypically, that is, slowly, with slight gas production or with no gas carbohydrates other than lactose, the most important of these being dextrose, sucrose, and mannitol. They may lack the capacity to produce indol, and other characteristic reactions may also be absent. Lewis[13] stresses the fact that one strain may be unstable for one test substance, for example, lactose, or for half a dozen; thus "a family tree of astounding complexity" may be developed.

In our laboratory we have used the following characteristics in the study of the paracolon bacilli: motility; growth on desoxycholate agar plates; the fermentation of lactose, dextrose, sucrose, and mannitol; the production of indol; and the utilization of sodium malonate as the sole source of carbon. The malonate broth of Leifson[14] has been used in place of citrate agar employed in many laboratories and has been found to be very satisfactory in differentiating Escherichia, which fail to utilize sodium malonate, from Aerobacter, which give a positive reaction. The fermentation of carbohydrates other than those listed has been investigated in various instances but has not proved helpful in the identification of these organisms. Agglutination tests with specific antiserum have been done in all cases where cultural similarity made the differentiation of the paracolon bacilli from the enteric pathogens difficult.

Using these characteristics, the paracolon bacilli which we have encountered in urine cultures have fallen into certain definite cultural types. In all instances the colonies on desoxycholate agar plates were colorless, indicating failure to ferment lactose promptly. On blood agar and on desoxycholate agar

plates, the colonies were usually considerably smaller than those of the typical coliform bacilli and occasionally were so atypical as to resemble those of streptococci. Of the other characteristics studied, we have found the most common variations to be as follows:

1. Failure to ferment lactose promptly; acid and gas produced after forty-eight hours to ten days incubation.
2. Delayed fermentation of lactose with acid and little or no gas.
3. Fermentation of dextrose with acid and no gas.
4. Failure to ferment sucrose.
5. Fermentation of sucrose with acid and no gas.
6. Failure to ferment mannitol.
7. Fermentation of mannitol with acid and no gas.
8. Failure to produce indol.
9. Loss of motility.

These differences define the cultural types listed in Table II.

One specific type of paracolon bacillus deserves special mention since its outstanding characteristic is not shown in Table II. This is the organism known as *Bacterium coli-mutabile* and originally described by Neisser[15] and Massini.[16] The recent literature contains excellent descriptions of this organism.[1-3, 10, 13] In lactose fermentation tubes *Bact. coli-mutabile* produces acid and a small amount of gas after forty-eight hours incubation, gas production increasing with prolonged incubation. Colonies on desoxycholate agar plates are colorless after forty-eight hours incubation. In three to five days, however, small, pink, secondary colonies, or papillae, appear growing on or out of the original colorless colonies. Subcultures from the basic, colorless colony ferment lactose slowly, while those from the colored, secondary colonies ferment lactose in twenty-four hours with acid and gas as do the typical coliform bacilli. These unstable strains may arise from organisms of the Escherichia, Aerobacter, or intermediate group. Stuart, Mickle and Borman[3] object to the name "coli-mutabile," since it suggests that these bacilli are all related to the Escherichia; they propose the term "papillae-forming coliforms" to describe these organisms. Although they occur infrequently and none are included in the series to be reported, we have isolated a number of this type of paracolon bacilli from urine cultures, and therefore their description has been included here.

As has been pointed out in Table II, the paracolon bacilli may culturally resemble Salmonella, Proteus, Shigella, or Eberthella. This resemblance is repeatedly stressed in the literature.[3-5, 7-12] In most instances, however, well-defined differences do exist which make cultural differentiation possible. The paracolon bacilli most frequently encountered in urine cultures are the late-lactose-fermenting varieties which produce acid and gas in the fermentation of carbohydrates. These may superficially resemble Salmonella or Proteus, although many of them are nonmotile. The fermentation of mannitol will eliminate Proteus, while a spreading growth on infusion agar will obviously confirm the identification. Salmonella and Eberthella will be eliminated by the production of indol or the utilization of sodium malonate. In the case of strains

TABLE II. CULTURAL CHARACTERISTICS OF PARACOLON BACILLI MOST FREQUENTLY ISOLATED FROM URINE CULTURES

LACTOSE	DEXTROSE	SUCROSE	MANNITOL	INDOL	MALONATE OR CITRATE	MOTILITY	CULTURALLY RESEMBLES	AGGLUTINATION* WITH SPECIFIC ANTISERUM
AG 48 hr. to 10 days	AG	AG/-	AG	+	-	+/-	Salmonella or Proteus	Negative
AG 48 hr. to 10 days	AG	AG	AG	-	+	-	Salmonella or Proteus	Negative
Negative	AG	AG/-	AG	+	-	+/-	Salmonella or Proteus	Negative
Negative	AG	AG	AG	-	+	-	Salmonella or Proteus	Negative
Acid only 48 hr. to 10 days	A only	A/-	A only	-	-	-	Shigella dispar	Negative
Acid only 48 hr. to 10 days	A only	A/-	A only	-	-	-	Shigella sonne	Negative
Negative	A only	-	A only	+	-	+/-	Shigella paradysenteriae	Negative
							Eberthella typhosa	Negative
Negative	A	-	-	+	-	-	Shigella ambigua	Negative
Negative	A only	-	-	-	-	-	Shigella dysenteriae	Negative

AG, Acid and gas.

A, Acid.

+/-, Positive or negative.

*Antiserum to be used will be that suggested by cultural similarity as indicated in Column 8. For example, a paracolon bacillus culturally resembling *Shigella dysenteriae* should be tested with *Sh. dysenteriae* antiserum.

resembling the Shigella, however, the complete cultural similarity often makes it necessary to set up agglutination tests with specific antiserum as indicated by the cultural reactions. If no agglutination occurs, the identity of the organism as a paracolon bacillus is strongly suggested.

The ease with which the paracolon bacilli may be confused with the true enteric pathogens cannot be overemphasized. Frequently they are erroneously classified as a member of the Salmonella or Shigella genera, although the resemblance is not usually sufficiently close to allow actual species identification. An illustration of the confusion which may result from the failure to recognize an organism as a paracolon bacillus can be found in the culture reports included in the history of a single patient on the gynecological service of the Johns Hopkins Hospital. This is the case of a woman with bilateral ureteral strictures and chronic pyelitis who has had fifty-two hospital admissions for cystoscopic treatments. For fourteen years all urine cultures were positive for *Escherichia coli*. During the succeeding ten years the urine culture reports in chronological order were as follows:

1. Unidentified gram-negative rods, lactose negative; dextrose, sucrose, and mannite fermented with acid and gas, gelatin negative, motility negative.
2. Nonmotile gram-negative rods with the sugars of *B. enteritidis*.
3. *B. coli*.
4. Nonmotile gram-negative rod, sugars of *B. enteritidis*.
5. *B. coli*.
6. Nonmotile gram-negative rod, sugars of *Salmonella enteritidis*.
7. Nonmotile gram-negative rod, acid and gas in dextrose, sucrose, and mannite, probably slow lactose fermenter.
8. *B. alkaligenes*.
9. Gram-negative rods, culturally *Proteus* (probably atypical strain).
10. *B. proteus*, probably atypical strain.
11. *B. proteus*, probable atypical strain.
12. Gram-negative rods, ferments the usual carbohydrates with acid and gas but does not ferment lactose (after eight days). Gelatin not liquefied.
13. Gram-negative rods which ferment dextrose and sucrose with acid and gas and do not ferment lactose. Do not liquefy gelatin.
14. Paracolon bacilli.
15. *E. coli* (in five succeeding cultures).
16. Atypical *Proteus*.
17. *Salmonella enteritidis*.
18. Paracolon bacilli (in six succeeding cultures).

These urine cultures were done by a number of bacteriologists in several laboratories over a period of twenty-four years. The last six cultures, all identified as paracolon bacilli, were studied in our laboratory and were definitely proved to be paracolon bacilli related to Escherichia. We do not feel it unreasonable to assume that the many unidentified or atypical oragnisms reported in previous years were also paracolon bacilli; indeed, the cultural reactions when included in the bacteriologic report confirm this point of view. In the light

of our experience with the variation of Escherichia and Aerobacter to paracolon bacilli in the urinary tract, as will be reported later, the assumption that these organisms were paracolon bacilli seems logical. If this assumption may be made, then certainly this case vividly illustrates the need for greater knowledge of the paracolon bacilli in medical bacteriology.

How may these errors in identification and the confusion of paracolon bacilli with Salmonella, Eberthella, Shigella, or Proteus be avoided? Knowledge of the various cultural characteristics of these aberrant coliform bacilli is, of course, essential, and a careful cultural and serologic study of the organisms will usually permit satisfactory differentiation. As far as the identification of paracolon bacilli from urine cultures is concerned, we have found certain points helpful in suggesting the identity of these organisms. A hint as to their possible identity may be gained from the fact that in these cases there is no clinical evidence to suggest infection with one of the Salmonella, Eberthella, or Shigella. Also, as will be discussed later in this report, paracolon bacilli most frequently occur in urine specimens together with typical coliform bacilli or from patients whose preceding urine cultures yielded Escherichia or Aerobacter. The finding of a lactose-negative, gram-negative bacillus under these circumstances is highly suggestive of a paracolon bacillus.

RELATION OF PARACOLON BACILLI TO URINARY TRACT INFECTIONS

A review of the literature on paracolon bacilli and on urinary tract infections has not yielded a great deal of information concerning the occurrence and significance of these organisms in urinary tract disease. Parr[1] states that "although practically all of the atypical coliform bacilli were first isolated from feces, they were early found in the urine." There he is referring to reports of Mair[17] and Wilson.[18] In 1906, Mair described organisms from two cases of cystitis which "agree with *B. coli*" with the exception of the fact that they produce no gas in lactose and glucose. These organisms Mair described as "paracolon bacilli." In 1908, Wilson reported six strains of gram-negative bacilli from urinary tract infections which resembled colon bacilli but differed in their failure to produce gas in the fermentation of carbohydrates. The classical work on the subject is that of Dudgeon and his co-workers in 1922 to 1927.[7, 19, 20] They report a series of acute urinary tract infections in which the etiologic agent was a "slow-lactose fermenting *B. coli.*" Since then writers on the subject of paracolon bacilli frequently mention in passing that these organisms may be responsible for infections of the urinary tract, referring to Dudgeon's work as authority for such statements and seemingly regarding the relationship as a probability rather than an established fact.[1-3, 12, 21] As far as actual isolation from urine cultures is concerned, only two recently reported instances could be found. Kennedy, Cummings, and Morrow[12] describe twenty-two strains of paracolon bacilli, four of which were isolated from urine. Cross[22] reports a "bacillus para coli" in a series of twenty-five organisms isolated from urinary tract infections. In a fairly comprehensive review of the recent literature on infections of the urinary tract made in connection with this and another report,[23] no other mention of paracolon bacilli in urine cultures could be found.

In the literature on urinary tract infections there is to be found, however, numerous reference to organisms described as unclassified gram-negative bacilli or as unidentifiable Salmonella, Eberthella, or Shigella which our experience suggests may well have been paracolon bacilli. For example, Marple[24] reports the isolation from urine from a case of chronic cystitis of a "gram-negative bacillus giving the sugar reactions of paratyphoid group but serologically unidentifiable." Cerf and Goldman[25] describe "gram-negative bacilli which do not show the sugar fermentations of known pathogens," and Stalker and Schulte[26] report the isolation from urine of four strains of "Shigella (species?)."

Hill, Seidman, Stadnichenko, and Ellis,[27] in their excellent study of two hundred gram-negative bacilli isolated from cases of genitourinary infection, report "sixteen miscellaneous cultures." These were identified as one Alkaligenes, five strains of Eberthella, nine Shigella, and one Salmonella. Of the fourteen Eberthela and Shigella strains one was not classified and the others were tentatively assigned to certain obscure species, although they did not correspond in all respects to recorded descriptions of these species. The bacillus considered *Salmonella paratyphi* fermented sucrose but in spite of this discrepancy was so classified because it was agglutinated by *S. paratyphi* immune serum. Since no recognized paracolon bacilli were included in this large series of 200 gram-negative bacilli and since, as will be discussed later, we have encountered these organisms with some frequency in urine specimens, it seems logical to presume that a number of these miscellaneous bacilli were paracolon bacilli of the type culturally resembling but not identical with certain Eberthella, Shigella, and Salmonella. The fact that the one sucrose-positive strain which was identified as *S. paratyphi* agglutinated with *S. paratyphi* antiserum does not eliminate the possibility of its being a paracolon bacillus, since Lommel,[28] using malachite green and safranin, suppressed lactose fermentation in cultures of colon bacilli and reports that three such experimentally produced lactose-negative strains became agglutinable with *Bacterium paratyphosum* B. antiserum.

Levine[29] has published a report on "Unclassifiable Gram-negative Rods in Urinary Infections." These organisms are described as failing to correspond to species described in Bergey's *Manual of Determinative Bacteriology* or as serologically differing from known species. Among the bacilli included in this report are strains which resemble *Esch. coli* but do not ferment lactose. Others resemble Shigella but do not agglutinate with known antiserum. Also described are "strains which are related to the Salmonella group biochemically but either form indol or are serologically distinct from the recognized Salmonella." In another report Levine[30] again refers to unclassifiable gram-negative bacilli, describes strains similar to those referred to previously, and includes a chart giving the cultural reactions of three types of atypical bacilli. The characteristics of a number of the organisms included in these publications strongly suggest that they may be paracolon bacilli; their description coincides with that of organisms of this group which we have isolated from urine specimens, and their identity as aberrant forms of coliform bacilli seems highly probable.

If such be the case and if a number of the unidentified bacilli reported from urinary infections are paracolon bacilli, then certainly attention need be directed to the characteristics of paracolon bacilli occurring in urine cultures, so that they may be recognized and correctly identified.

As has been stated, we have isolated a number of paracolon bacilli from urine cultures. The specimens from which these organisms were cultured were received from women patients on the gynecological and obstetrical services of the Johns Hopkins Hospital. The organisms were isolated by our routine procedure as described in Schaub and Foley.[31] They produced colorless colonies on desoxycholate agar, indicating their failure to ferment lactose promptly and were isolated from such colonies, and their characteristics as tabulated in Table II were studied. Agglutination tests with appropirate antiserum as indicated by the cultural reactions have been carried out in all instances where cultural reactions alone were insufficient for differentiation.

A study of the incidence and significance of these paracolon bacilli in the female urinary tract has been made. The series includes all cases from both the gynecological and obstetrical services, for the year 1945, in which these organisms were found. The incidence of paracolon bacilli in this series is given in Table III. As shown here, 100 strains of paracolon bacilli were isolated, eighty-six from patients on the gynecological service and fourteen from obstetrical cases. These represent 3.02 per cent of the total number of urine cultures received in the laboratory during this year and 4.3 per cent of the positive cultures obtained.

TABLE III. OCCURRENCE OF PARACOLON BACILLI IN URINE SPECIMENS FROM WOMEN PATIENTS ON GYNECOLOGICAL AND OBSTETRICAL SERVICES

SERVICE	TOTAL NUMBER OF URINE SPECIMENS	NUMBER OF POSITIVE CULTURES	NUMBER OF PARACOLON BACILLI	PERCENTAGE OF TOTAL URINE CULTURES	PERCENTAGE OF POSITIVE CULTURES	NUMBER OF PATIENTS
Gynecology	2,486	1,847	86	3.06	4.6	46
Obstetrics	822	452	14	1.7	3.09	11
	3,308	2,299	100	3.02	4.3	57

The 100 strains of paracolon bacilli were isolated from fifty-seven different patients as shown in Table III. Histories were available for fifty-one of these patients, and these histories were carefully reviewed for evidence of urinary tract infection. The results of this study are given in Table IV. Here it is shown that of the forty-patients on the gynecological service eight were without symptoms of urinary tract infection, five with no white blood cells, and three with white blood cells in catheterized urine specimens. Eleven patients showed symptoms of cystitis; eight were diagnosed as acute and three as chronic infections. Symptoms of pyelitis were present in twenty-one instances, the infection being acute in eleven cases, and ten patients having chronic disease. Thus it is seen that in a total of thirty-five, or 87.5 per cent, of the patients on the gynecological service signs or symptoms of urinary tract infection were present. Of the eleven obstetrical cases, six showed no signs or symptoms of urinary tract infections, while five, or 45.5 per cent, showed symptoms of acute pyelitis.

TABLE IV. RELATION OF PARACOLON BACILLI TO URINARY TRACT SYMPTOMS

SERVICE	WITHOUT SYMPTOMS		SYMPTOMS OF CYSTITIS			SYMPTOMS OF PYELITIS			TOTAL NUMBER OF CASES
	WITHOUT WHITE BLOOD CELLS	WITH WHITE BLOOD CELLS	ACUTE	CHRONIC	TOTAL	ACUTE	CHRONIC	TOTAL	
Gynecology	5	3	8	3	11	11	10	21	40
Obstetrics	6	–	–	–	–	5	–	5	11
Total number of cases	11	3	8	3	11	16	10	26	51
Percentage of total number of cases	21.5	5.8	15.6	5.8	21.5	31.3	19.6	50.9	

The number of chronic infections in which paracolon bacilli were involved deserves comment. Thirteen cases, three of chronic cystitis and ten of chronic pyelitis, a total of 25.5 per cent, are included in this series. This contrasts with the reports of Dudgeon and associates[7, 19, 20] who emphasize the acute nature of the disease and the rapid, complete recovery of the patients included in the series of cases they reported.

RELATION OF PARACOLON BACILLI TO ESCHERICHIA AND AEROBACTER IN THE URINARY TRACT

We have been impressed with the frequency with which paracolon bacilli either were found following the isolation of Escherichia or Aerobacter in a previous urine specimen from the same patient, occurred together with normal lactose-fermenting strains of these organisms, or were followed by the isolation of typical coliform bacilli. Histories of the patients from whom paracolon bacilli have been isolated were therefore studied for information concerning the association of the aberrant coliform bacilli with typical Escherichia and Aerobacter. The results of this study are contained in Table V. Here it is shown that in our series of fifty-one patients for whom histories were available, in twenty-seven, or 52.9 per cent, of the cases the paracolon bacilli either were preceded by, occurred with, or were followed by Escherichia or Aerobacter. Association with Escherichia occurred with far greater frequency, 45.9 per cent, than with Aerobacter, 7.8 per cent. This association was frequently very close, since in a number of cases the paracolon bacilli not only were preceded by the isolation of Escherichia or Aerobacter but also occurred with and were followed by typical coliform organisms. The cases in which this occurred are represented in Table V by the figures in parentheses.

Twenty-four, or 47 per cent, of the cases showed no proved association with Escherichia or Aerobacter. It should be noted, however, that in twenty of the twenty-four cases with no proved association between the aberrant and typical coliform organisms little opportunity to demonstrate such an association was provided. There was only one recorded urine culture on these twenty patients, and that one culture was the one in which paracolon bacilli occurred in pure culture. Thus, of the thirty-one cases in which more than one culture was

TABLE V. ASSOCIATION OF PARACOLON BACILLI WITH TYPICAL STRAINS OF ESCHERICHIA AND AEROBACTER

PARACOLON BACILLI ASSOCIATED WITH	RELATION OF PARACOLON BACILLI TO ESCHERICHIA OR AEROBACTER	ISOLATED FROM PATIENTS WITH						NUMBER OF STRAINS	PERCENTAGE OF TOTAL NUMBER OF STRAINS
		NO SIGNS OR SYMPTOMS OF U.T.I.	WHITE CELLS ONLY	ACUTE CYSTITIS	CHRONIC CYSTITIS	ACUTE PYELITIS	CHRONIC PYELITIS		
Escherichia	Preceded by	1	–	2	2	5	5	15	29.4
	Occurred with	1	1	–	(1)*	2	2 (2)	6	11.7
	Followed by	–	–	–	(2)	2 (3)	(5)	2	3.9
	Total	2	1	2	2	9	7	23	45.9
Aerobacter	Preceded by	–	–	1	–	2	1	4	7.8
	Occurred with	–	–	–	–	(2)	(1)		
	Followed by	–	–	–	–	–	(1)		
	Total	0	0	1	0	2	1	4	7.8
Number of strains associated with Escherichia and Aerobacter		2	1	3	2	11	8	27	52.9
Number of strains with no proved association with Escherichia or Aerobacter		9	2	5	1	5	2	24	47
Total number of strains		11	3	8	3	16	10	51	
Percentage associated with Escherichia or Aerobacter		18.1	33.3	42.5	66.6	68.7	80	52.9	

*Figures in parentheses represent cases included in figures directly above.

taken and an opportunity offered to demonstrate association with Escherichia or Aerobacter, twenty-seven, or 87.1 per cent of these cases showed this association.

Table V also demonstrates that association of paracolon bacilli with Escherichia or Aerobacter occurs more frequently in chronic than in acute infections; in 80 per cent of the cases with chronic pyelitis as compared with 68.7 per cent of the cases of acute pyelitis and in 66.6 per cent of the patients with chronic cystitis as compared with 42.5 per cent of the cases of acute cystitis. In patients showing white cells only, the incidence of this association was 33.3 per cent, and in patients with no signs or symptoms of urinary tract infection only, 18.1 per cent. The higher incidence in patients with chronic disease may well be explained by the fact that in such cases it is possible to study the infection over a relatively long period of time and to obtain numerous cultures on such patients. Thus an opportunity is afforded to establish an association between these organisms. In acute cases there is frequently only one specimen taken, and thus there is no evidence that an Escherichia or Aerobacter isolated from this one culture was followed by a variant form or, if a paracolon bacillus was isolated, that it was preceded or followed by typical coliform bacilli.

DISCUSSION

The incidence and pathogenicity of paracolon bacilli in the female urinary tract has been established by the data presented in Tables III and IV. A possible explanation of the presence of these atypical coliform bacilli is afforded by the evidence of the association of these organisms with Escherichia and Aerobacter in 52.9 per cent of the cases as shown in Table V. As has been pointed out, if only the cases in which more than one culture had been taken are considered, for only in cases with a number of cultures was ample opportunity to prove this association provided, then in 87.1 per cent of the cases the paracolon bacilli were preceded by, occurred with, or were followed by typical Escherichia or Aerobacter. This close association suggests that in at least a number of cases the organism initiating the infection was an Escherichia or Aerobacter and that the paracolon bacilli arose from these typical coliform bacilli through variation in the urinary tract. We were interested in determining, if possible, if this theory were tenable and, if so, what factors or conditions might be responsible for this variation.

A review of the literature has not proved fruitful in suggesting what factors may be involved in the production of paracolon bacilli. That they are aberrant forms of Escherichia and Aerobacter is well established,[1-5, 8-13] and the nature of the factors influencing this variation has been the subject of considerable speculation. Kline[12] suggests that paracolon bacilli are coliform organisms which may have become modified through the influence of unfavorable environment. A number of investigators[1, 3, 4] have pointed out that the incidence of paracolon bacilli in feces from patients with gastroenteritis is considerably higher than normal. Stuart, Mickle, and Borman[3] express the opinion that the presence of the aberrant coliform organisms in such stool cultures "may

represent an alteration in the normal fecal flora, possibly as a result of a physiologic disbalance in the individual." Again in regard to the isolation of paracolon bacilli from the infected intestinal tract, Dudgeon and Pulvertaft[7] state that conditions in the intestines may be such that the rapid multiplication of the slow-lactose-fermenting organisms is allowed. Parr[1, 9] has demonstrated that in stored feces there is a marked change in the flora resulting in the disappearance of typical Escherichia and more especially Aerobacter and a marked increase in the slow-lactose-fermenting variants (paracolon bacilli). This change, Parr suggests, is due to the degradation of ordinary, normal coliform organisms.

As far as we have been able to ascertain, the variation of Escherichia and Aerobacter to paracolon bacilli has never been demonstrated in the urinary tract. We feel, however, that the evidence presented in Table V suggests that such a variation does take place. Since the urinary tract is not the normal habitat of the coliform bacilli, this change in environment may account for the variation. It may also be possible that some factor provided by the infected state of the urinary tract may be responsible for the change from typical to aberrant coliform organisms.

In vitro studies and experiments have furnished considerable conflicting evidence concerning variation in coliform organisms. A number of investigators have been unable to bring about the change from lactose-fermenting to the lactose-negative form. Parr and Robbins[11] express the opinion that "the prompt and typical fermenter of that sugar (lactose) is a well-stabilized form in respect to that test." Stewart[33] and Hall[34] failed to observe the production of lactose-negative from lactose-fermenting strains. Hershey and Bronfenbrenner[35] were unable by serial transfers in synthetic media containing sodium succinate as the sole source of carbon to cause the variation of typical *Esch. coli* to the lactose-negative form, although variants of paracolon bacilli which had reverted to prompt lactose fermenters changed in this medium with the resultant production of lactose-negative strains.

Other investigators have been able to demonstrate the production of aberrant coliform bacilli from typical Escherichia and Aerobacter. Stuart, Mickle, and Borman[3] report the finding of nonlactose fermenting variants in two strains of typical coliform bacilli after two years on agar slants. These variants were antigenically identical with the parent strains. Kriebel[5] also reports that of 120 strains of Escherichia stored for several months on agar slants nineteen gave rise to lactose-negative variants. Lommel[28] suppressed lactose fermentation using media containing malachite green and safranin, and Penfold[36] obtained lactose-negative strains from coliform bacilli grown on media containing chloracetic acid. This work contributes little information concerning the factors influencing this variation, but these reports do demonstrate that the variation does take place, that paracolon bacilli may arise naturally from coliform organisms (that is, on stored agar slants), or that they may be produced artificially by growth on media containing certain substance.

In our series of cases not only did Escherichia and Aerobacter precede and occur with paracolon bacilli, but in ten instances *Esch. coli* was found following the isolation of paracolon bacilli in pure culture in a previous specimen. This suggests that the aberrant strains may revert in the urinary tract to typical, prompt-lactose-fermenting varieties. This reversion is well known in *Bact. coli-mutabile*[1-4, 10, 11, 13] in which secondary colonies of lactose-fermenting strains are produced invariably by colonies of the slow-lactose-fermenting form. Investigators have been able to bring about this reversion in other paracolon bacilli,[3, 5, 12, 13, 37] and we have been able to accomplish this in a number of instances. Therefore, it seems probable that in the urinary tract not only may the aberrant paracolon bacilli be produced from Escherichia and Aerobactor but also that these paracolon bacilli may, in turn, give rise to typical coliform organisms. Indeed, in eight instances in our series the occurrence of paracolon bacilli in pure culture was both preceded and followed by the isolation of Escherichia.

Although we believe that our evidence suggests that the origin of paracolon bacilli in the urinary tract may, in some cases at least, be by way of variation from typical coliform bacilli, our study has yielded no evidence of the factors concerned in this variation. It seemed possible that chemotherapy might play a role in bringing about this change. The presence in the urine of high levels of sulfonamides or penicillin in patients being treated with these substances might possibly be a factor favoring variation. A study of the histories of the patients included in this series did not confirm this theory. In the fifty-one cases reviewed, paracolon bacilli were isolated before the initiation of chemotherapy in forty-six instances. In only five cases did these organisms appear after treatment, once each after the administration of sulfadiazine, sulfasuccidine, and penicillin and in two patients following sulfamerazine therapy. These figures seem to eliminate such therapy as a factor in the variation of coliform organisms in the urinary tract.

The length of time that the coliform bacilli had been present in the urinary tract was also considered as a possible factor. A study of the histories revealed that the paracolon bacilli were isolated at intervals varying from two days to fourteen years following the first isolation of Escherichia or Aerobacter. Thus prolonged growth in the urinary tract was eliminated as an important factor in favor of the variation from typical to aberrant coliform organisms.

The cause of this apparent variation and the factors influencing it remain unknown. However, we believe that the evidence which we have presented does suggest that in the infected urinary tract conditions are present which influence and favor the variation of Escherichia and Aerobacter and result in the appearance of paracolon bacilli in urine cultures.

SUMMARY

1. Paracolon bacilli appear to be aberrant forms of Escherichia and Aerobacter which differ from the typical coliform organisms in their failure to ferment lactose promptly with acid and gas. Other variations may also

occur, so that a large number of culturally different paracolon bacilli may be encountered. The common variations which we have found in paracolon bacilli from urine cultures have been listed, and the cultural types frequently isolated are given in Table II.

2. Culturally paracolon bacilli may resemble Proteus, Salmonella, Shigella, and Eberthella and are frequently mistaken for members of these genera. They may usually be differentiated biochemically, but in some instances serologic tests are necessary.

3. In one year, 100 strains of paracolon bacilli were isolated from urine specimens from fifty-seven women patients, representing 3.02 per cent of the total number of urine cultures received in the laboratory during 1945 and 4.3 per cent of the positive cultures.

4. Fifty-one histories were studied to determine the significance of paracolon bacilli in the female urinary tract. In 50.9 per cent of the cases there were symptoms of pyelitis; in 21.5 per cent, symptoms of cystitis. White blood cells only were found in 5.8 per cent of the patients, and 21.5 per cent showed no signs or symptoms of urinary tract infection.

5. In 52.9 per cent of the cases the paracolon bacilli were associated in the urinary tract with either Escherichia or Aerobacter. If only the instances in which more than one culture was taken are considered, then this association between typical and aberrant coliform bacilli occurred in 87.1 per cent of the cases.

6. It has been suggested that these paracolon bacilli may, in a number of cases at least, be the result of variation in the urinary tract, that in such cases the original infecting agent was a typical Escherichia or Aerobacter, and that, under conditions as yet undetermined, variation occurred in these typical coliform organisms with the resultant appearance of paracolon bacilli in urine cultures.

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CONJOINED ACTION OF PENICILLIN AND BACTERIOPHAGES

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IN THE successful healing of an infectious disease, many agencies ordinarily participate. General medication may well be directed to establish those qualities in the internal nutrient media of the body, particularly the blood and lymph, which will favor the successful activity of the human cells and discourage or inhibit the activities of the parasitic organisms, with due consideration of the natural biologic properties and mechanisms for defense and offense inherent in host cell and parasite. The relationships are ordinarily complex rather than simple.

The malignant type of infection with staphylococcus in which the bacterial invader has been able to master the local defenses so as to gain and persist in the circulating blood, septic staphylococcemia, is one example of disease in which there is urgent need of an alteration in quality of the blood and lymph so as to oppose more effectually the infectious agent and perhaps to tip the scale in the conflict in favor of the human host. Even after this has succeeded to the point of eliminating the bacteria from the general circulation, there often remain metastatic localizations of the infection, particularly in bone, kidney, or skin. Suppression of the infection in these focal lesions may require the prolonged maintenance of the special healing qualities in the circulating blood. Many internal remedies have been used, from time to time, in the attempt to improve the blood so as to aid in overcoming the staphylococcus. Various preparations of arsenic, bismuth, mercury, and tin have come down to us from the older therapeutics, and they still have their place. In the more modern era, however, some of the synthetic organic chemicals, particularly sulfathiazole, and some of the biologic agents, bacterial vaccines, bacteriophages, and penicillin, have come into the foreground. The conjoined action of the last two, namely bacteriophage and penicillin, has been remarkably promising in our experience during the last few years.

Our earlier studies of staphylococcemia[1] were concerned especially with the production of potent bacteriophages in such form that they could be administered by intravenous injection in large amounts without serious untoward effects and also with the practical therapeutic use of these bacteriophages in the treatment of septic staphylococcemia, its complications and sequelae, in experimental animals and in human patients. Bacteriophage therapy was employed alone and also in conjunction with neoarsphenamine in the subacute and chronic staphylococcemias. With the advent of sulfathiazole, the use of

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this chemical in suitable combination with bacteriophages seemed to offer advantages, in spite of its toxic effects in older patients. The combination was particularly helpful in the early fulminant stage of staphylococcemia. Ordinarily, we found it best to discontinue the sulfathiazole after the first or second week. The newer biologic agent, Fleming's penicillin, offers greater advantages because it can be produced in a potent form which is almost entirely free from toxic action upon the human host even when used by intravenous injection continuously for many months. This agent alone inhibits the growth of many strains of staphylococcus. However, there are strains of this organism which are naturally resistant to penicillin, and some of the susceptible strains are able to acquire a high resistance to this agent after a period of adaptation.

Complete understanding of biologic phenomena is perhaps still beyond our mental capacity, but it now seems well established that the staphylococcus becomes resistant to penicillin in part by elaborating a substance or substances, to which the general name of penicillinases has been given, which neutralize, inhibit, or destroy the bacteriostatic and bactericidal properties of the penicillin. Doubtless all staphylococci produce these substances in some amount, and the strains which have greater resistance to penicillin produce greater amounts of them. Naturally penicillin-resistant strains of bacteria may in part account for the occasional disappointment in penicillin therapy of fulminant staphylococcemia, while the acquired resistance would seem to be operative especially in the more chronic infections, such as persistent osteomyelitis.

Before discussing individual clinical examples of conjoined therapy, some of the work with cultures in vitro may be mentioned. Himmelweit[2] working in the laboratory of Sir Alexander Fleming at St. Mary's Hospital, London, has observed that a strain of staphylococcus moderately resistant to both penicillin and bacteriophage became much more susceptible to destruction when exposed to the action of these two biologic agents in combination. Repetition of Himmelweit's experiments in our laboratory, using our own bacteriophages and cultures obtained from septic patients in our own series, has resulted in essential confirmation of his observations.

Gram-negative bacteria of the coli group are ordinarily resistant to the action of penicillin. However, when combined with bacteriophage, the penicillin exerts a synergistic effect in vitro against cultures of colon bacilli which seems to be analogous to the similar effect on the staphylococcus.

In one instance a strain of colon bacillus (Nbl) obtained in a blood culture was found resistant to penicillin, so that no inhibition of growth was evident in broth containing as much as 5,000 units penicillin per 100 c.c. of medium. When, however, this colon bacillus was grown in broth containing only 50 units penicillin per 100 c.c., the filtrate of this flourishing culture showed peculiar properties. When added to newly inoculated broth, this filtrate caused complete lysis of the Nbl strain at two hours and at four hours followed by secondary clouding at twenty-four hours, and this phenomenon was observed consistently in subsequent serial filtrations, even without further

addition of penicillin. Therefore, a native bacteriophage, latent in the original Nbl strain, had revealed itself as lytic for this strain in the presence of small amounts of penicillin and exhibited lytic effect not only in the culture tube containing penicillin but, after attaining potency in this medium, was able to induce temporary lysis in subsequent serial propagation without penicillin in the medium. Evidently the presence of the penicillin favored the bacteriophage in its action against the host bacterial cell, so that the latent bacteriophage, originally so feeble as to escape recognition, acquired potency against its host cell. These observations are of theoretical interest for the student of bacteriophagy and also of practical significance for the rapid production of a bacteriophage to be used in treatment of a desperately ill patient.

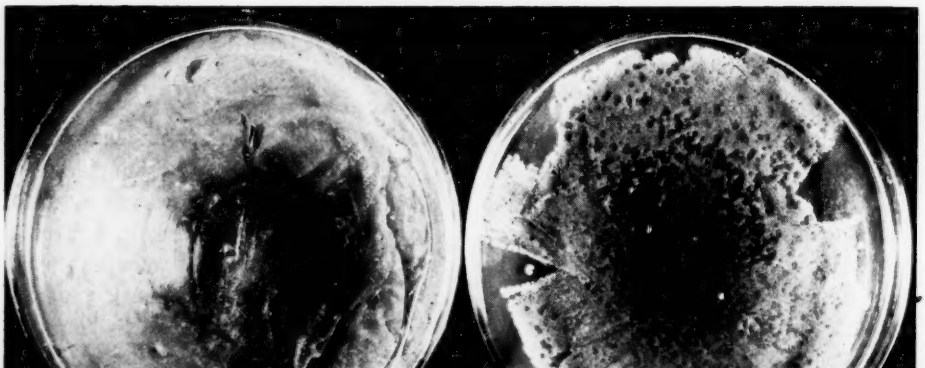

Fig. 1.—Photograph of plate cultures on agar, showing at the left a mass culture of the original Nbl strain and at the right a precisely analogous mass culture of this Nbl strain after the activity of its latent bacteriophage had become enhanced by propagation in the presence of penicillin and serial filtrations.

The protocol of one of these experiments is shown in Table I. The filtrate of the respective preceding generation was used in Generations 2 to 5, with penicillin in Generations 1 to 3 but no penicillin in Generations 4 and 5. In each instance the degree of clouding was estimated by comparison with the control tube recorded as 4 plus at the particular time. In Fig. 1 is shown a photograph of an agar plate on which had been spread 0.05 c.c. of suspension of coli culture, Nbl strain, with subsequent incubation for eighteen hours. Here the growth was confluent without recognizable sign of bacteriophage effect. On the other agar plate there was spread 0.05 c.c. of a precisely analogous suspension of coli culture, Nbl strain, of Generation 5 in the experiment recorded in Table I, with subsequent incubation for eighteen hours. Here the confluent bacterial growth presented the moth-eaten appearance due to the formation of numerous plaques by action of the bacteriophage.

The pathogenic streptococci of the hemolyticus group and of the salivarius group are for the most part susceptible to the bacteriostatic action of penicillin

TABLE I

GENERATION	1	2	3	4	5	CONTROL
Meat infusion broth	10.0 c.c.	10.0 c.c.	10.0 c.c.	10.0 c.c.	10.0 c.c.	10.0 c.c.
Fresh coli culture	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.
Penicillin units	5	5	5	None	None	None
Filtrate 0.5 c.c.	None	Gen. 1	Gen. 2	Gen. 3	Gen. 4	None
Incubation at 37° C.						
Growth cloud						
Two hours	++++	++	0	0	0	++++
Four hours	++++	0	0	0	0	++++
Twenty-four hours	++++	++++	++++	++++	++++	++++

and usually resistant to bacteriophages. *Streptococcus fecalis* is a notable exception, as we[3, 4] have previously reported. This organism, which is not infrequently the infecting microbe in bacterial endocarditis, is ordinarily very resistant to penicillin and at the same time susceptible to lysis by suitable bacteriophages. More recently we have met with another type of streptococcus, closely resembling *Streptococcus salivarius* but relatively less susceptible to penicillin and capable of lysis by bacteriophage in vitro. Infections with organisms of this type present an opportunity for therapeutic use of penicillin and bacteriophages in combination.

Clinical application of these principles has already been made. Detailed case reports cannot be presented here, but brief summaries in regard to a few patients treated at the Post-Graduate Hospital may serve to illustrate the procedures and the results and perhaps may encourage others to use penicillin and bacteriophages in combination when indicated.

M. R., a 21-year-old white man, was admitted to the hospital for the eighth time on April 8, 1944, with persistent sinus and ulcerations of upper left tibia and chronic osteomyelitis of fourteen years' duration. The detailed record of treatment in the hospital for the period of about two months is too voluminous for presentation at this place, but a brief summary may be of interest. The first intravenous injection at 4:45 P.M. April 8 contained 5 c.c. specific staphylococcus bacteriophage and 5,000 units of calcium penicillin. Subsequently 2 c.c. specific staphylococcus bacteriophage and 5,000 units penicillin calcium were given every two hours by intramuscular injection, 6:30 P.M. April 8 to 6:30 A.M. April 9; then at 8:29 A.M. an intravenous injection of the same dose was given. At 10:25 A.M. April 9, the bacteriophage was increased to 5 c.c. and the penicillin calcium to 10,000 units, given every two hours by intravenous injection during the day from about 8:30 A.M. to 4:30 P.M. and by intramuscular injection during the night. On April 10, because of general malaise, nausea, and headache, the dose was changed at 10:24 A.M. to bacteriophage 2 c.c. and penicillin calcium 20,000 units during the day and bacteriophage 5 c.c. with penicillin calcium 20,000 units during the night, continued to April 18 at 10:21 A.M. At this time the penicillin dose was reduced to 10,000 units every two hours and on April 23 at 8:28 A.M. to 5,000 units. Bacteriophage doses were varied somewhat from 2 to 5 c.c. On May 3 the night doses were reduced to 1 c.c. and the doses during the day continued at 2 c.c. At this time the buttocks were quite sore from the

many intramuscular injections, and the openings on the leg were practically healed. The patient was allowed to be out of bed. The penicillin was discontinued after 2:25 P.M. May 4, and the bacteriophage continued by intravenous injection 2 c.c. only twice daily to May 17. A small area of ulceration still remained, and so on May 17 the injections every two hours of bacteriophage 2 c.c. and penicillin calcium 10,000 units were started again. The ulcer was also treated locally with undiluted bacteriophage and penicillin 600 units per cubic centimeter for irrigation. The frequent injections were again discontinued on June 11, and only the bacteriophage in dose of 2 c.c. was continued twice daily to June 15. The last crust separated on June 11 leaving a smooth pink epidermis.*

TABLE II

	BACTERIOPHAGE (C.C.)	PENICILLIN (UNITS)
	October 9	
2:10 P.M.	5 I.V.	10,000 I.V.
3:10 P.M.	10 I.V.	10,000 I.V.
4:15 P.M.	15 I.V.	10,000 I.V.
5:18 P.M.	20 I.V.	
6:15 P.M.		10,000 I.M.
8:15 P.M.		10,000 I.M.
10:15 P.M.		10,000 I.M.
	October 10	
12:15 A.M.		10,000 I.M.
2:15 A.M.		10,000 I.M.
4:15 A.M.		10,000 I.M.
6:15 A.M.		10,000 I.M.
8:10 A.M.	10 I.V.	10,000 I.V.
10:13 A.M.	10 I.V.	10,000 I.V.
12:15 P.M.	10 I.V.	10,000 I.V.
2:07 P.M.	10 I.V.	10,000 I.V.
4:15 P.M.	10 I.V.	10,000 I.V.
6:15 P.M.		10,000 I.M.
8:15 P.M.		10,000 I.M.
10:18 P.M.		10,000 I.M.
	October 11	
12:15 A.M.		10,000 I.M.
2:15 A.M.		10,000 I.M.
4:15 A.M.		10,000 I.M.
6:15 A.M.		10,000 I.M.
8:09 A.M.	10 I.V.	10,000 I.V.
	5 local irrigation	
10:15 A.M.	10 I.V.	10,000 I.V.
12:14 P.M.	10 I.V.	10,000 I.V.
2:15 P.M.	10 I.V.	10,000 I.V.
4:13 P.M.	10 I.V.	10,000 I.V.
	October 12	
12:15 A.M.		10,000 I.M.
2:15 A.M.		10,000 I.M.
4:15 A.M.		10,000 I.M.
6:15 A.M.		10,000 I.M.
8:10 A.M.	10 I.V.	10,000 I.V.
	5 local irrigation	
10:10 A.M.	10 I.V.	10,000 I.V.
12:10 P.M.	10 I.V.	10,000 I.V.
2:15 P.M.	10 I.V.	10,000 I.V.
3:14 P.M.	10 I.V.	10,000 I.V.
	October 13	
8:09 A.M.	9 I.V.	
	1 local irrigation	
10:13 A.M.	10 I.V.	
12:19 P.M.	10 I.V.	
2:10 P.M.	10 I.V.	

E. M., a 54-year-old white woman, secretary to a surgeon, was admitted Oct. 9, 1944, with right facial carbuncle of 5 days' duration. On the day of admission it presented brawny edema of the face, threatening extension to the right orbit, and the temperature reached 102.0° F. on this day. After taking blood for sedimentation rate and blood culture and pus from the carbuncle for culture, the patient was treated with bacteriophage and penicillin as shown in Table II. Note that the injections were given intravenously during the day and intramuscularly during the night. The patient was discharged at 4:30 P.M. Oct. 13 in excellent condition and even escaped a visible scar.

*This patient was seen on July 29, 1946. His condition at this time was excellent. There had been no recurrence of ulceration.

C. S., a 36-year-old white man was admitted Dec. 3, 1943, with mitral endocarditis due to *Staphylococcus aureus*, the growth of which was inhibited in vitro by 2 units penicillin per 100 c.c. of medium. After treatment with bacteriophage and penicillin to Feb. 28, 1944, the patient was discharged March 18 and has continued to receive bacteriophage injections at intervals. He has remained well and was active at work in May, 1944. This case has been reported.[5]

S. D., a 64-year-old white woman, was admitted to the hospital Dec. 31, 1944, partly disoriented, with temperature of 104.6° F. and harsh systolic murmur over the aortic area, irregular pulse, and extra systolic contractions every three or four beats. Blood cultures taken Dec. 31, 1944, and Jan. 2, 3, and 4, 1945, all developed growth of *Staphylococcus aureus*, which was not appreciably inhibited by 500 units of penicillin per 100 c.c. of medium. It was also moderately resistant to the available bacteriophages. The patient was given penicillin and staphylococcus bacteriophage every two hours from January 2 to February 26, and the bacteriophage was continued twice daily to May 18. She was discharged June 16, 1945, and has remained in good condition. The long record of this patient has been prepared for separate publication.

L. C., a 69-year-old white woman, was admitted to hospital at 4:45 P.M. Aug. 3, 1944, with a large ventral hernia which had become strangulated and gangrenous. There was central crepitation and firm brawny induration about the periphery. She was given 20,000 units of penicillin by intramuscular injection at 5:30 P.M. and then taken to the operating room. The central portion had already ruptured to permit escape of gas. At the operation this spontaneous opening was enlarged, and the perforated loop of colon was drawn out of the wound and supported by a heavy glass rod through the mesocolon and surrounded by several pads to prevent its retraction. The patient was returned to bed in a critical condition. Penicillin, 20,000 units, was given by intramuscular injection at 7:30 P.M. and every two hours during the night. Culture of the exudate from the wound yielded *Clostridium welchii, Escherichia* coli, and two types of anaerobic streptococci. At 9:30 A.M. August 4, 0.2 c.c. of coli bacteriophage was given along with the 20,000 units penicillin by intramuscular injection, and this dose was continued every two hours to August 21. At this time she was allowed to dangle her feet. The colostomy continued to function well. On September 23 the portion of transverse colon involved in the colostomy was fully resected, with end-to-end anastomosis of the colon and anatomical repair of the hernia. She was discharged in good condition on Nov. 20, 1944.

M. N., a white woman, born Dec. 8, 1882, had a gastroenterostomy performed in 1934 and a subtotal gastrectomy on June 21, 1945. During a stormy postoperative period of six weeks with temperature range from 99.4 to 105.4° F., she received numerous intravenous infusions of dextrose, sulfadiazine, whole blood, blood plasma, and amigen. On August 3 the patient had an unusually severe chill with rise in temperature to 106.0° F. A specimen of blood taken at 4:00 P.M. on this day yielded growth of approximately 1,900 colonies of *Esch. coli* per cubic centimeter of the patient's blood. Penicillin and coli

bacteriophage were then administered to September 2, and the bacteriophage continued to Sept. 19, 1945, when the patient was discharged in good condition. The record of this patient is too long for presentation here.

S. A., a 47-year-old white man, was admitted Aug. 29, 1945, and after careful preoperative preparation the splenic flexure of the colon was resected on September 4 to remove an annular carcinoma. Subsequently the temperature rose and there was a chill on September 6, with signs of pneumonia. Sulfadiazine and penicillin were administered without favorable effect. On September 17 he was disoriented, and there were bubbles of gas in the subcutaneous tissues easily detected by crackling over the chest wall and left upper extremity. In this grave situation stock coli bacteriophage and penicillin were given by injection every two hours. Following abdominal paracentesis on September 20, the peritoneal cavity was irrigated twice daily with penicillin and coli bacteriophage, and after spontaneous rupture of the recently healed surgical wound, the fecal fistula at this site was irrigated twice daily with the penicillin-bacteriophage mixture. There was extensive soiling of the general peritoneal cavity with intestinal contents and free communication between the drain in the paracentesis and the opening of the spontaneous fecal fistula. The penicillin and the bacteriophage were discontinued on November 16, and the patient was discharged to his home on November 19. On May 20, 1946, he appeared to be in excellent condition. The record of this patient is in preparation for presentation elsewhere.

P. M., a 34-year-old white man, was admitted Jan. 23, 1944, and discharged Nov. 16, 1944. He had bacterial endocarditis with both *Str. fecalis* and *Str. salivarius* found in cultures of his blood. He was treated with enterococcus bacteriophage, penicillin, and sodium bismuth thioglycollate. The case report has already appeared.[6] We may add that the patient remained in good condition, actively engaged in business in June, 1946.

J. P., a 27-year-old white man, was admitted July 21, 1945, by transfer from another hospital where he had been treated for bacterial endocarditis with sulfadiazine and penicillin over a period of four months, during which time the bacterial infection of the blood stream continued. The streptococcus of his blood culture required 8 units penicillin per 100 c.c. to inhibit macroscopic growth. The patient was treated with penicillin, bacteriophages, and sodium bismuth thioglycollate and was finally discharged in good condition on Feb. 11, 1946. He was actively engaged in business in June, 1946. The long record of this patient is in preparation for separate presentation.

COMMENT

The recent additions to the therapeutic agents available for internal medication in infectious diseases have improved in a marvelous fashion the outlook for success in dealing with these conditions. The biologic agents, penicillin, and the bacteriophages are not antagonistic to each other, and in many instances they can be shown to exert a synergistic effect upon the bacterial culture in the test tube. This combined action in the test tube may be designated as a potentiating, conjoined, or synergistic effect. The precise mechanisms

concerned require further elucidation. For practical purposes it is significant that this combined action is also manifest in the clinical use of these agents in treating otherwise malignant infections, particularly those due to staphylococci, colon bacilli, and selected members of the streptococcus group.

The brief notes in regard to nine patients serve to indicate the sort of results which have been achieved by the diligent use of these agents in combination. Some of these cases have already been reported elsewhere in more detail. Others of them are of such practical interest that detailed case reports may be expected to appear in the future. Meanwhile, it is hoped that physicians confronted with problems of this sort may be encouraged to undertake the therapeutic programs which have sometimes succeeded in our hands.

SUMMARY

1. Cultures of staphylococcus moderately resistant to penicillin and to bacteriophages may be more effectively inhibited in their growth by a combination of these agents. Similar observations have been made on cultures of colon bacilli and some types of streptococci.

2. Severe types of infection with staphylococcus, such as chronic osteomyelitis, facial carbuncle, and staphylococcal endocarditis have responded to combined therapeutic use of penicillin and bacteriophages.

3. Intestinal perforation associated with general peritonitis and bacteremia has been successfully treated with coli bacteriophage and penicillin.

4. Bacterial endocarditis due to streptococci relatively resistant to penicillin has responded favorably to treatment with penicillin and suitable streptococcus bacteriophage.

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THE INTERPRETATION OF THE WEIL-FELIX AGGLUTINATION TEST IN ROCKY MOUNTAIN SPOTTED FEVER

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AFTER the significance of the Weil-Felix agglutination in epidemic typhus was demonstrated,[1] an attempt was made to ascertain whether this reaction occurred in another rickettsial disease, namely, Rocky Mountain spotted fever. While Kelly,[2] in 1923, obtained negative results with a proteus OX-19 strain in serum specimens from eight patients with Rocky Mountain spotted fever, Kerlee and Spencer,[3] in 1923, reported that the serum from some patients developed agglutinins with OX-19 strains which were far in excess of those encountered in control specimens. In four cases of Rocky Mountain spotted fever, where single specimens were obtained during the febrile period or in early convalescence, titers of from 1/320 to 1/2560 were observed. In one case, where serial specimens were available, a definite rise in titer with an OX-19 strain was recorded. In 1930, Spencer and Maxey,[4] employing both OX-19 and OX-2 strains in a series of four patients from whom serial specimens were examined, observed one patient who showed a high OX-19 (1/1280) and a low OX-2 titer (1/160) early in the disease, followed by a low OX-19 (1/80) and a higher OX-2 titer (1/640) in early convalescence. The second patient showed a high OX-19 titer (1/2560) and no agglutinins with an OX-2 strain; the third patient developed low titers for both OX-19 (1/40) and OX-2 (1/160); and the fourth patient showed a high OX-19 (1/2560) and a high OX-2 (1/640) titer. Davis and Parker,[5] in 1932, studied a series of patients from whom single specimens were examined and observed a high OX-19 and a low OX-2 relationship or a low OX-19 and a high OX-2 titer. This was also observed in patients from whom serial specimens were examined. These authors stressed the fact that relatively high titers with an OX-2 strain were occasionally encountered. In 1934, Davis, Parker, and Walker[6] again reported a series of patients in whom various OX-19 : OX-2 relationships were observed, and the same authors,[7] in 1938, reported two patients from whom rickettsial strains were isolated who showed high OX-2 and low OX-19 titers. It had thus been shown that an OX-19 agglutination could be obtained in cases of Rocky Mountain spotted fever and that occasionally higher titers with an OX-2 strain were present.

In 1933, Felix[8] prepared a table (Table I) representing the main and group agglutinins in different varieties of typhus. Data obtained from this table have been extensively quoted. According to these findings, it would appear that the OX-19 titer in Rocky Mountain spotted fever is lower than that found

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TABLE I. MAIN AND GROUP AGGLUTININS IN DIFFERENT VARIETIES OF TYPHUS[8]

	OX-19	OX-2	OX-K
Epidemic typhus	+++	+	- -
Endemic typhus	+++	+	- -
Scrub typhus	- -	- -	+++
Rocky Mountain spotted fever	+	+	+

in either epidemic or murine typhus and that the OX-2 and OX-K agglutination is equal in titer to that found for the OX-19. Since these results are at variance with those observed in this laboratory, it was considered worth while to present out data. While specific serologic reactions are now available for the diagnosis of Rocky Mountain spotted fever,[9] the Weil-Felix agglutination test still remains an important laboratory aid. Its value, however, depends upon the proper interpretation of the results obtained.

The data presented in Table II represents the results of Weil-Felix tests obtained from a series of cases of Rocky Mountain spotted fever occurring in the United States Army personnel. The laboratory diagnosis was established in each case either by the isolation of the infectious agent from the blood of the patient or by the demonstration of the presence of specific complement-fixing antibodies of Rocky Mountain spotted fever.[9]

The Weil-Felix tests, using controlled nonmotile proteus strains of OX-19, OX-2, and OX-K, were performed according to the technique described in *Diagnostic Procedures and Reagents.*[10] The complement fixation tests were performed according to the method previously described.[9]

An analysis of these data would indicate that the most frequent proteus agglutination pattern in cases of Rocky Mountain spotted fever is a high OX-19 and a lower or negative OX-2 titer (eleven cases). This is followed in frequency by a high OX-2 titer and a lower or negative OX-19 agglutination (four cases), a low or negative OX-19 and a negative OX-2 (three cases), and finally, one case in which equal titers were observed. These results are comparable to those reported by Spencer and Maxey[4] and Davis and Parker.[5-7]

It should be observed that 21 per cent of the patients in this series showed a high OX-2 titer and a lower or negative OX-19 agglutination. This combination has not been reported as occurring in either epidemic or murine typhus nor have we found it in a series of such patients from whom serial specimens were examined.[11, 12]

While the response to the proteus antigens may vary in individual cases, a common pattern or combination of results can be described as being characteristic for each rickettsial disease. The usual reactions observed in epidemic and murine typhus are characterized by a rise in OX-19 titer, which in certain instances may reach high levels. In these cases the OX-2 titer, when it occurs, is lower than the OX-19, and the OX-K is negative. This conforms to the results as recorded by Felix.[8] In Rocky Mountain spotted fever the OX-19 titer may be high and the OX-2 lower or negative. In these cases the OX-19 titer may reach levels comparable to those ordinarily found in either epidemic or murine typhus. When the OX-19 titer is lower or negative and the OX-2 higher, we are justified in suspecting a case of Rocky Mountain spotted fever. Indeed,

TABLE II. WEIL-FELIX AGGLUTINATION IN CASES OF ROCKY MOUNTAIN SPOTTED FEVER

CASE	DAY OF ILLNESS	WEIL-FELIX		COMPLEMENT FIXATION ROCKY MOUNTAIN SPOTTED FEVER
		OX-19	OX-2	
1	21	0	1/1280	0
	31	0	1/320	0
	40	1/80	1/80	+
2	7	0	0	+
	23	0	0	+
3	10	1/160	1/2560	+
	14	1/160	1/1280	+
4	4	1/40	0	0
	5	1/80	0	0
	13	1/2560	1/80	+
5	17	1/2560	1/640	0
	35	1/320	1/160	+
6	7	0	0	0
	10	0	1/160	0
	24	0	1/640	+
	51	1/80	1/80	+
7	17	1/640	0	+
	34	1/1280	0	+
	65	1/640	0	+
8	9	0	0	0
	16	0	0	+
	18	1/160	1/80	A/C
9	5	0	0	0
	18	1/320	1/320	+
	45	1/40	1/80	+
10	9	0	1/40	0
	12	1/160	1/1280	+
11	4	0	0	+
	9	1/640	1/160	+
12	12	0	0	0
	16	1/2560	1/160	+
	21	1/1280	1/160	+
	31	1/1280	0	+
13	6	0	0	0
	9	0	0	+
	18	1/40	0	+
14	5	0	0	0
	12	1/640	0	+
15	7	1/160	0	0
	14	1/640	0	0
	15	1/640	0	-
	21	1/640	0	+
16	13	1/40	0	0
	25	0	0	+
17	8	0	0	-
	13	0	0	-
	23	1/2560	0	+
	37	0	0	+
18	9	1/320	1/40	+
	28	1/2560	1/80	+
19	7	0	0	0
	13	1/640	1/320	0
	20	1/1280	1/640	0
	140	-	-	+

the presence of an OX-2 agglutination titer of 1/320 or higher would lead us to suspect a case of Rocky Mountain spotted fever. However, since the Weil-Felix proteus agglutination is considered a nonspecific test, the final laboratory diagnosis will depend upon the isolation of the infectious agent or the demonstration of specific antibodies.[13] In scrub typhus the OX-19 and OX-2 agglutination are negative, and a rise in OX-K titer is usually, but not always, present.[14, 15] A rise in OX-K titer was formerly regarded as being characteristic of scrub typhus alone, but Zarafonetis, Ingraham, and Berry[16] have recently shown that this can occur in louse-borne relapsing fever. The proteus agglutination reactions in "Q" fever are all negative.[17, 18]

TABLE III. USUAL WEIL-FELIX AGGLUTINATION REACTIONS OBSERVED IN RICKETTSIAL DISEASES

	OX-19	OX-2	OX-K
Epidemic typhus	++++	+	0
Murine typhus	++++	+	0
Scrub typhus	0	0	++++
"Q" fever	0	0	0
Rocky Mountain spotted fever	++++	+	0
	+	++++	0

The results, as recorded in Table III, are suggested as representing the usual Weil-Felix agglutination results occurring in cases of epidemic and murine typhus, scrub typhus, "Q" fever, and Rocky Mountain spotted fever.

SUMMARY

The usual Weil-Felix agglutination found in Rocky Mountain spotted fever is characterized by a high OX-19 and a lower or negative agglutination with an OX-2 strain and a negative OX-K. In these cases the OX-19 titer may reach levels equal to those found in either epidemic or murine typhus. The next most frequent combination is a high OX-2 titer and a lower or negative OX-19 agglutination. Since this grouping has not been described as occurring in cases of epidemic or murine typhus, we are justified in suspecting a case of Rocky Mountain spotted fever when it is present. The final laboratory diagnosis, however, will depend upon the isolation of the infectious agent or the demonstration of a rise in titer of specific antibodies. A table is suggested as representing the usual Weil-Felix agglutination reactions observed in rickettsial diseases.

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SULFONAMIDES AND DARK ADAPTATION

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FOLLOWING the description by Bucy[1] of a case of optic neuritis resulting from the administration of sulfanilamide, various visual disturbances have been described following sulfonamide therapy.

Since 1939, a number of transient myopias have been reported in the literature.[2-13] The majority of these cases were due to sulfapyridine, while sulfathiazole and sulfadiazine seemed innocuous in this respect. The pathogenesis of these disturbances is obscure, but in 62 per cent of the reported cases the myopia was brought on by usual therapeutic doses (from 2 to 10 Gm.) of the responsible agent. Mydriasis usually accompanied the myopia, and treatment with atropine and homatropine could not be shown definitely to alter its course. However, when administration of the drug was stopped, vision gradually returned to normal.

Cataracts, retinitis, reduction of the visual fields, blurred vision, and changes in accommodation have variously been reported in isolated cases of patients receiving sulfonamides. Alvaro,[14] Rosenthal,[15] and Reynolds, Evans, and Walsh[16] have noted the effects of sulfonamides on visual fields, visual acuity, light and color sense, accommodation, muscle balance. For the most part, all these authors found some reduction in the width and length of visual fields, as well as a decreased power of accommodation. None of these authors, however, reported any marked change in visual acuity, and, so far as we have been able to ascertain, the literature does not contain a reference to the effects of sulfonamides on dark adaptation.

It is common knowledge that some persons who require therapy with sulfonamides (pilots, bombardiers, motorists, locomotive engineers) have been told not to follow their profession during a course of these drugs. It has been taken for granted, it is presumed, that the reason for such a statement has had to do with some decrease in the visual efficiency of such individuals when they have been so treated. For this reason we are reporting our experiments on the effects of three commonly used sulfonamides upon dark adaptation.

For the determination of the dark adaptation time, a Feldman adaptometer† was used. For each test the following technique was routinely adopted: The subjects were requested to avoid bright lights before the test. For ten minutes they sat quietly in a semidarkened room. The test was performed in a dark room. For three minutes the subjects looked at a bright light. This was then turned off, and the time required to identify the direction of an

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†American Optical Company.

oblong test light of uniform blue-violet color was determined as the dark adaptation time. The brightness of the lights was about 2.3 lamberts for the exposure light and log 6.18 $\mu\mu$ lamberts for the test light.* For the identification of the test light, the subject sat at reading distance from it and was requested to direct both eyes toward a "fixation point" in order that the threshold be taken on a predominantly rod area.

Using this method, the dark adaptation time of eighteen normal volunteers of both sexes† was determined before, during, and after medication with sulfathiazole, sulfadiazine, and sulfanilamide.

The subjects were divided into three groups of six. Group A received 4 Gm. of sulfathiazole daily for three days and Group B a similar dosage of sulfadiazine. In Group C, two subjects received 4 Gm. of sulfanilamide, but in four cases the medication period was shortened because of toxic manifestations such as nausea, vomiting, and general discomfort.

The drugs were taken in four doses at the following times: 8:30 and 11:30 A.M., 2:00 and 4:00 P.M. Adaptometer tests were made three times daily at fifteen minute intervals between 5:00 and 7:00 P.M. before and after (control period), as well as during, the medication period. Blood samples were taken during the medication period on the first and third days immediately after the completion of adaptation time determinations. Free and total blood sulfonamide concentration was determined by the method of Bratton and Marshall.[19]

TABLE I

GROUP	DRUG	DARK ADAPTATION TIME IN SECONDS		BLOOD SULFONAMIDES (FREE MG. %)
		CONTROL PERIOD	MEDICATION PERIOD	
A	Sulfathiazole	49 ± 20	57 ± 22	6.0 ± 1.3
B	Sulfadiazine	42 ± 22	37 ± 19	7.6 ± 1.4
C	Sulfanilamide	44 ± 23	67 ± 46	7.0 ± 0.8

The results are summarized in Table I. As shown by the standard deviations, the values for dark adaptation times in each group were somewhat variable. This was true not only for the data treated as a whole in each group but also for the determinations made on each single subject. This is not surprising considering the multiple influences, psychologic and other, that play upon the subject endeavoring to spot the test light and estimate its direction in minimum time. With practice, however, results became more uniform, and the time required for dark adaptation decreased. This trend was apparent in all three groups.

Under the conditions of our experiments, no group revealed a statistically significant difference between the adaptation times of the control and medication periods. However, in two subjects of Group C, the dark adaptation times were consistently elevated during the medication period. It appears difficult to evaluate at present the significance of these changes. It should be

*This was erroneously printed as log 1.6μ lambert in the abstract (Federation Proc. 4: 135, 1944).

†We wish to thank the medical students (now physicians) at the University of Vermont and the technicians of Mary Hitchcock Hospital, Hanover, New Hampshire, who were subjects for this experiment.

noted that all values fall well within the range of five minutes characteristic of normal night vision when tested with this apparatus (Feldman[20]). They also fall within the more narrow range of normal values given by Hecht and Mandelbaum[21] for test lights of the same brightness.

As the subjects used in these experiments were in normal health throughout the experiment and were mostly trained to observe and note their own feelings, it is interesting to report the symptoms evidenced by them during their medication period.

In Group B, with the exclusion of two slight headaches, no untoward symptoms were reported. All subjects of Group A receiving sulfathiazole witnessed paresthesia of the tongue; this took the form of tingling and numbness and sometimes disturbances of taste. Subjects of Group C were most affected of all. Without exception they complained of headaches, nausea, dizziness, and general malaise throughout the entire medication period. One subject vomited and discontinued the drug after the first day. In three others, although there was no vomiting, the symptoms were so pronounced that they led to the discontinuing of the drug after the second day. Four cases reported paresthesias of feet and legs. In all three groups mental slowness and some disturbance of memory was a frequently reported symptom.

SUMMARY

1. Results on eighteen healthy individuals indicate that neither sulfathiazole, sulfadiazine, nor sulfanilamide significantly alters dark adaptation time as measured by the Feldman adaptometer.

2. There is an indication, however, that those subjects which received sulfanilamide exhibited enough increase in their dark adaptation times to suggest a possibility that this sulfonamide may have a direct effect upon dark adaptation.

3. These experiments would not corroborate the general opinion that persons taking small doses of sulfonamides daily need necessarily be concerned about visual acuity or efficiency.

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LIVER FUNCTION TESTS IN NEUROSYPHILITIC PATIENTS WITH INDUCED VIVAX MALARIA OF PACIFIC AND MEDITERRANEAN ORIGIN

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IN AN earlier paper[1] it was reported that only mild, transient disturbances of liver function were noted in patients with naturally acquired, relapsing vivax malaria of Pacific origin. All of these soldiers had been given quinacrine promptly to terminate their acute attacks. In this paper are presented the findings in 138 soldiers with neurosyphilis whose induced malarial fever was terminated with quinacrine only after they had had on the average eight to fourteen paroxysms and about twenty days of parasitemia.[2]

SUBJECTS AND METHODS

The subjects were 138 soldiers with neurosyphilis who had completed at least one standard course of antisyphilitic chemotherapy. Prior to inoculation with vivax malaria of Pacific or Mediterranean origin by the bites of laboratory-bred, infected, domestic, anopheline mosquitoes or by the intravenous injection of five to 7 c.c. of blood containing trophozoites, tests of liver function were made. The tests were repeated during the course of the malaria and at varying times following termination of the malaria with quinacrine (2.8 Gm. in 6 days). The patients had an average of forty hours of fever over 104° Fahrenheit.

The following tests were performed: bromsulfalein, cephalin flocculation, galactose tolerance, intravenous hippuric acid, icterus index, serum bilirubin, and urine urobilinogen. The details of the methods used have been given in an earlier paper.[1]

RESULTS

Bromsulfalein Test.—The bromsulfalein test was carried out in 138 neurosyphilitic patients (Table I). The amount of dyestuff injected was 5 mg. per kilogram of body weight, and retention of up to 4 per cent at forty-five minutes was considered normal.[1] Prior to fever therapy 138 patients were tested, and 117, or 85 per cent, gave normal findings. Twenty-one men had abnormal values including ten with 6 per cent retention, eight with 8 per cent, one with 10 per cent, one with 12 per cent, and one with 40 per cent. Following fever therapy (from 10 to 100 days later) sixty-six of these 138 men were re-examined, and in fifty-eight men, or 88 per cent, the results were normal. The eight men with abnormal values included two with 8 per cent retention, one

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TABLE I. BROMSULFALEIN TESTS IN PATIENTS WITH NEUROSYPHILIS BEFORE, DURING, AND AFTER MALARIAL FEVER THERAPY

RELATION TO FEVER THERAPY	BEFORE	DURING (15 TO 40 HOURS OF FEVER)	AFTER (10 TO 100 DAYS LATER)
Number of patients	138	18	66
Number of tests	138	18	66
Patients with retention of 6 per cent or greater			
Number	21	15	8
Per cent	15	83	12

with 10 per cent, two with 12 per cent, one with 16 per cent, one with 32 per cent, and one with 40 per cent. Of the 138 patients only eighteen were tested before, during, and after fever therapy. Fourteen of these men had normal values in the pre-fever study, while three had 6 per cent retention and one 8 per cent. During fever therapy fifteen of these men had abnormal values with those in ten ranging between 12 and 40 per cent. Following therapy fifteen of the eighteen patients had normal values, while of the remaining three with abnormal values one had 10 per cent retention, one had 12 per cent, and one had 32 per cent.

Cephalin Flocculation Test.—A series of 256 sera from forty patients (Table II) were tested with the Wilson antigen, and 181 sera from thirty-six of these same patients were tested with the Difco antigen. Before inoculation with malarial parasites and from inoculation to the finding of the first positive smear for malaria, no 3 or 4 plus results were obtained. During the next four days 12 per cent of the results with the Wilson antigen and 15 per cent with the Difco antigen were 3 or 4 plus. After the fifth day there was a sharp increase

TABLE II. CEPHALIN FLOCCULATION TESTS IN PATIENTS WITH NEUROSYPHILIS BEFORE AND DURING MALARIAL FEVER THERAPY

TIME OF TESTS WITH RELATION TO MALARIA	PERCENTAGE OF 3 AND 4 PLUS TESTS	
	WILSON ANTIGEN (256 TESTS IN FORTY PATIENTS)	DIFCO ANTIGEN (181 TESTS IN THIRTY-SIX PATIENTS)
Before inoculation	0	0
Inoculation to first positive smears	0	0
During days of positive smears		
1 to 4	12	15
5 to 7	35	33
8 to 11	70	94
12 to 14	89	96
15 to 18	97	100
19 to 21	100	100

in positive results, so that within a period of from fifteen to eighteen days with the Difco antigen and from nineteen to twenty-one days with the Wilson antigen all had a 3 or 4 plus test which persisted during the next week in those individuals who were tested. No tests were performed following fever therapy. It is noteworthy that in the present studies the sensitivity of the Wilson and Difco antigens was about the same, whereas in the previous study[1] the Difco antigen at that time was more sensitive.

Galactose Tolerance Test.—The galactose tolerance test was performed on 106 patients prior to malarial therapy, and only one of these excreted over the accepted highest normal value of 3 Gm. of galactose in five hours. No tests were done during fever therapy. Following fever therapy thirty-one men were retested, and all findings were normal. It so happened that the patient with a slightly abnormal value before fever therapy was not re-examined after therapy.

Intravenous Hippuric Acid Test.—The intravenous hippuric acid test was performed on twenty-five patients. Results were reported in terms of hippuric acid in the urine specimen voided one hour after the intravenous injection of 1.77 Gm. of sodium benzoate. A value below 0.80 Gm. was considered abnormal.[1] Prior to fever therapy all values were normal. After a minimum of fifteen hours of temperature of not less than 104° F., three of twenty-two men who were retested had abnormal findings. From thirty to eighty days after disappearance of fever, twelve of the twenty-five men were retested, and ten were normal. The other two men had abnormal values during fever, and on this occasion following fever one still remained abnormal. In two of the three men with abnormal results during the period of fever therapy, there was also definite evidence of bromsulfalein retention, namely 36 and 40 per cent, respectively. Following fever therapy the bromsulfalein values were normal in these two patients.

TABLE III. ICTERUS INDEX, SERUM BILIRUBIN, AND URINE UROBILINOGEN IN PATIENTS WITH NEUROSYPHILIS BEFORE, DURING, AND IMMEDIATELY AFTER MALARIAL FEVER THERAPY

		PERIOD OF PARASITEMIA		PERIOD OF SIX DAYS' QUINACRINE TREATMENT	
	SEVEN DAYS BEFORE FIRST POSITIVE SMEAR	SEVEN DAYS BEGINNING WITH FIRST POSITIVE SMEAR	SEVEN DAYS BEFORE QUINACRINE TREATMENT	FIRST THREE DAYS	LAST THREE DAYS
Icterus Index					
Number of patients	12	32	32	32	32
Number of tests	12	44	57	53	34
Per cent tests > 8	8	12	38	47	15
Serum Bilirubin					
Number of patients	13	32	32	32	32
Number of tests	13	45	63	54	33
Per cent tests > 0.50	7	7	17	24	12

Urine Urobilinogen

	SEVEN DAYS BEFORE FIRST POSITIVE SMEAR	SEVEN DAYS BEGINNING WITH FIRST POSITIVE SMEAR							SEVEN DAYS BEFORE QUINACRINE TREATMENT							SIX DAYS OF QUINACRINE TREATMENT						
		1	2	3	4	5	6	7	7	6	5	4	3	2	1	1	2	3	4	5	6
Number of patients	32	18	21	21	25	21	21	22	20	18	22	24	26	25	25	25	24	28	23	27	23
Number of tests	92	18	21	21	25	21	21	22	20	18	22	24	26	25	25	25	24	28	23	27	23
Per cent tests > 1:20	0	6	5	14	4	14	19	41	25	28	36	38	35	40	40	36	46	25	9	15	6

TABLE IV. LIVER FUNCTION TESTS IN THIRTY-TWO NEUROSYPHILIS

PATIENTS	INITIAL STUDIES					DURING PERIOD OF PAROXYSMS							
						FIRST WEEK			SECOND WEEK			SECOND TO FOURTH WEEK	
	ICTERUS INDEX	BILIRUBIN	BROM-SULFALEIN	HIPPURIC ACID	GALACTOSE	ICTERUS INDEX	BILI-RUBIN	UROBIL-INOGEN	ICTERUS INDEX	BILI-RUBIN	UROBIL-INOGEN	BROM-SULFALEIN	HIPPURIC ACID
1	6	0.10	6%		Neg.	6	0.10	1:5	6	0.15	1:20		
2	15	0.70	2%		0.56		0.50	1:10	7	0.35	1:50		
3	5	0.25		0.97	0.87							14%	0.61
4	5	0.10	4%		1.25	7	0.30	1:5	5	0.20	1:5		
5	8	0.40	Neg.		1.32	6	0.30	1:5	8	0.35	1:20		
6	8	0.35	Trace		1.11		0.30	1:5	10	0.35	1:50		
7	9	0.40	2%		0.32		0.15	1:5	9	0.35	1:5		
8	8	0.40	4%		0.94	6	0.20	1:5	12	0.90	1:50		
9	12	0.40	2%	1.05	0.18								
10	6	0.20	2%	0.90		6	0.20	1:100			1:10		
11	8	0.30	2%		0.58			1:10	8	0.30	1:5		
12	6	0.30	2%		1.02	6	0.25	1:10	20	2.00	1:100		
13	7	0.30	6%		0.63	6	0.25	1:10	12	0.80	1:100	32,40%	
14	7	0.30	2%		2.13	7	0.20	1:20	9	0.70	1:500	12, 8%	
15	6	0.30	Trace		1.63	7	0.20	1:5	7	0.30	1:50		
16*	5	0.10	Trace		0.45	14	0.50	1:50	50	8.00	1:100		
17*	7	0.20	2%		0.71			1:50	18	1.00	1:100		
18*	7	0.30	2%		0.56	6	0.10	1:5	6	0.25	1:50	40%	
19	7	0.40	6%	0.92								32, 8%	0.8
20	6	0.20	Trace		1.74	7	0.20	1:10	9	0.35	1:200		
21	7	0.30	2%		0.49	6	0.20	1:20	12	0.75	1:10		
22	8	0.40	Trace		0.82		0.20	1:10		0.10	1:50		
23	6	0.40	Neg.		1.04			1:20	9	0.65	1:50		
24	6	0.20	Trace	0.77									
25	7	0.45	Trace	0.67	1.09			1:5	10	0.65	1:50	4%	0.67
26	7	0.20	2%		0.64	14	1.00	1:20	12	0.65	1:50		
27	9	0.20	Trace		0.63	10	0.30	1:500	10	0.65	1:500		
28	9	0.40	2%	1.05								6,6%	0.2
29	6	0.20	2%		0.62	6	0.20	1:20		0.20	1:200		
30	6	0.40	2%	0.83								32,8%	0.5
31	9	0.70		0.89	0.79	7	0.45	1:5					0.6
32	20	2.50		0.87	1.88							0%	0.8
32†	32	32	29	10	27	17	21	25	21	23	24	14	7
‡	22	9	10	20	0	18	5	16	67	48	71	86	71

The results shown in this table are expressed as follows: icterus index, in units; bilirubin, in milligra the dye per kilogram body weight; hippuric acid, in grams excreted in the urine in one hour after the in the giving of 40 Gm. of galactose by mouth; urobilinogen, highest dilution of urine giving a positive test.

*Developed clinical jaundice.

†Number of tests.

‡Per cent abnormal.

Icterus Index and Serum Bilirubin Concentration.—Sera were taken from thirty-two patients for determination of the icterus index and bilirubin concentration of the same specimens (Table III). A value of over 8 units was considered as abnormal for the icterus index and over 0.5 mg. per 100 c.c. for the serum bilirubin concentration.[1] In the seven-day periods before and following the first positive smear, a maximum of 12 and 7 per cent of abnormal values was reached for the icterus index and serum bilirubin, respectively, with a rise to 38 and 17 per cent of abnormal values in the last seven days before termina-

ATIENTS WITH SPOROZOITE INDUCED PACIFIC MALARIA

CLINICAL FINDINGS FIRST TO FOURTH WEEK				DAYS OF QUINACRINE TREATMENT									17 TO 70 DAYS AFTER QUINACRINE TREATMENT		
	LIVER	G. I. SYMPTOMS		FIRST			SECOND			FOURTH					
HIPPURIC ACID	TENDERNESS	NAUSEA	VOMITING	ICTERUS INDEX	BILIRUBIN	UROBILINOGEN	ICTERIC INDEX	BILIRUBIN	UROBILINOGEN	ICTERUS INDEX	BILIRUBIN	UROBILINOGEN	BROMSULFALEIN	HIPPURIC ACID	GALACTOSE
	No	Yes	Yes	7	0.35	1:5	7	0.30		4	0.10	1:10	8%		
	No	Yes	No										2%		1.12
	No	Yes	Yes										Trace	0.99	
	No	Yes	Yes	8	0.25	1:5	6	0.20	1:5			1:5	Neg.		
	No	Yes	No	10	0.35	1:5	8	0.35	1:10	7	0.10	1:5	Trace		1.37
	No	Yes	Yes				15	0.75		9	0.35	1:20	2%		0.86
	No	No	No	7	0.25	1:200	8	0.40	1:50	7	0.25	1:5	4%		1.05
	Yes	Yes	Yes				30		1:100	15	2.00	1:200	4%		0.65
	No	Yes	Yes										Trace	1.06	
	No	Yes	No	8	0.35	1:5	6	0.30	1:5	5	0.10	1:5			
	Yes	No	No	8	0.40	1:5	6	0.30	1:5	6	0.10	1:5	Trace		0.69
	No	Yes	Yes	12	0.80	1:5	10	0.50		7	0.20	1:5	4%		0.43
	No	Yes	Yes	12	0.60	1:20	8	0.30	1:50	7	0.35	1:5	10%		0.16
	Yes	Yes	Yes	10	0.45	1:5	11	0.60	1:50	6	0.10	1:5	Trace		1.33
	No	Yes	Yes	10	0.40	1:20	10	0.65	1:200	7	0.40	1:20	Neg.		0.66
	Yes	Yes	No	50	7.00	1:50	50	4.50		30	1.40	1:100	2%		0.20
	Yes	Yes	Yes			1:250	65	4.90				1:100	2%		1.01
	Yes	Yes	Yes	40	3.50	1:500	16	0.65	1:100			1:20	2%		
	No	No	No										2%		
	No	Yes	No			1:5	10	0.55	1:5			1:5	Trace		2.34
	Yes	Yes	Yes	6	0.30		8	0.45	1:5	6	0.20	1:5	2%		0.76
	No	Yes	No			1:100			1:200	16	1.00	1:100	4%		0.57
	No	Yes	No	10	0.35	1:20	10	0.45	1:20	7	0.20	1:5	Trace		0.76
	No	No	No											0.97	
	No	Yes	Yes	9	0.55	1:50	9	0.80	1:20	7	0.20		Neg.	0.96	
	No	Yes	No	12	0.85	1:5	12	0.90	1:100	7	0.20	1:10	4%		0.18
	No	Yes	Yes	8	0.35	1:50	12	0.55	1:500	6	0.10		2%		
	No	No	No										4%		
	No	Yes	Yes	7	0.30	1:5	6	0.10	1:5	6	0.10	1:5	2%		
	No	Yes	Yes										2%		
	No	Yes	Yes											0.58	
	No	Yes	Yes										40%	0.59	
	32	32	32	18	18	20	22	21	18	19	19	21	29	6	17
	25	84	59	56	33	35	60	48	50	21	16	19	10	33	0

100 c.c. of blood; bromsulfalein, in per cent retention at forty-five minutes after the giving of 5 mg. of
ous injection of 1.77 Gm. of sodium benzoate; galactose, in grams excreted in the urine in five hours after

tion of fever by quinacrine. The increase carried over into the first three days of quinacrine treatment during which abnormal values rose still further to 47 and 24 per cent, respectively. However, during the next three days abnormal values dropped to 15 and 12 per cent, respectively.

Urine Urobilinogen.—The test for urine urobilinogen was performed on fresh morning specimens. A positive test in a dilution greater than 1:20 was considered to be abnormal and is herein referred to as a positive test. Examinations of the urine were carried out in the same thirty-two men on whom icterus

index and serum bilirubin concentrations were determined. In the week prior to the appearance of the first positive smear for malaria, ninety-two tests were made on the group and all were negative. In a daily study for seven days from the finding of the first positive smear, there was an increase in positive tests from 6 to 41 per cent. This value was not exceeded in the seven days prior to termination of quinacrine therapy, and during six days of such treatment the positive values fell gradually to 6 per cent.

Serial Tests of Liver Function With Clinical Correlation.—It was not possible to carry out all the tests of liver function in all 138 patients before, during, and after fever therapy. However, fairly complete studies were made in thirty-two patients. The results of these studies are shown in Table IV, together with certain clinical findings in these cases.

All of the patients were considered to be good risks for fever therapy. None presented clinical evidence of hepatic disease prior to treatment, except that one patient showed a slightly, and another a moderately, elevated serum bilirubin (other tests in these patients were within normal limits).

Of twenty-nine patients adequately followed with liver function tests during the course of fever, twenty-one or 72 per cent showed results that deviated slightly or moderately from normal by one or more tests. Of these twenty-one patients sixteen showed hepatic enlargement, six showed hepatic tenderness, nineteen had nausea, thirteen had vomiting, and three developed clinical jaundice during the period of malarial activity. Two patients showed some hepatomegaly but no manifest alteration in liver function. In all instances except three (Patients 1, 13, and 32) the observed changes in liver function returned to normal after completion of malarial therapy. In only one of the three (Patient 32) was the residual abnormality such as to make the prognosis with respect to liver disease a guarded one.

At this hospital, of 172 patients treated with vivax malaria of foreign origin, eight men, or 5 per cent, developed clinical jaundice. In all eight cases the liver was enlarged and tender, bile was present in the urine, and the van den Bergh test was delayed or direct. The clinical picture was that of an acute hepatitis and not that of icterus caused solely by excessive hemolysis of red cells. Following fever therapy there was no clinical evidence of hepatic disease. In six of these patients on whom bromsulfalein tests were performed after fever therapy, the results were normal. In all instances termination of fever with quinacrine and the institution of general supportive measures (intravenous solutions of glucose, high carbohydrate and protein diets, and extra vitamins) resulted in a fairly prompt disappearance of the jaundice and a return of abnormal liver function tests to normal. In none was there evidence of permanent hepatic damage.

DISCUSSION

These patients with neurosyphilis formed a desirable group in which to study liver function because: (1) most of them had completed a standard course of chemotherapy; (2) an insectary for the raising of *Anopheles quadrimaculatus* mosquitoes for sporozoite inoculation was available; (3) it had

previously been shown in this laboratory that South Pacific vivax malaria, which was to be used therapeutically in many of these patients, disclosed some evidence of transient disturbance in liver function during recurrent attacks but gave little or no indication of permanent hepatic dysfunction; and (4) the patients could be observed both clinically and by laboratory tests over long periods of time, so that the data gathered could be evaluated and compared with current reports of therapeutic malaria of American origin.

It has been stated by Fredricks and Hoffbauer[3] that the postmalarial death of a 46-year-old woman under their observation sharply focused their attention on the scant evaluation in the literature of hepatic function in therapeutic malaria. These authors investigated thirty-one patients with neurosyphilis inoculated intravenously with benign tertian malaria. No manifest liver disease was noted prior to malarial therapy. Hepatic function was evaluated by several types of tests. These authors felt that all of their patients adequately studied showed some evidence of hepatic dysfunction but that the abnormalities reported were not presumed to indicate irreversible hepatic damage and the occurrence of such changes did not constitute a reason for avoiding the use of a well-tried and effective therapeutic agent. Kopp and Solomon[4] studied the liver function in fifty-one patients with neurosyphilis (previously given tryparsamide over long periods of time) treated with malarial fever, artificial fever, or fever induced by typhoid vaccine. Their impression was that following such therapy some alteration of liver function may develop but in most instances is only of mild degree.

In our series of patients with neurosyphilis, Pacific and Mediterranean vivax malaria was induced both by sporozoite and blood inoculation. In general, the results of our serial studies of liver function using malaria of foreign origin are similar to those cited[3, 4] in the use of malaria of American origin. Before fever therapy a small percentage of our patients showed slightly abnormal values with the bromsulfalein and serum bilirubin tests but with no other tests. The abnormalities were possibly related either to previous antisyphilitic chemotherapy or to syphilis itself. During fever therapy bromsulfalein retention occurred in most patients studied with values ranging up to 40 per cent. Abnormal values were also observed during fever therapy with the cephalin flocculation test (all 3 or 4 plus) and with the icterus index, serum bilirubin, and urobilinogen tests. After termination of fever therapy with quinacrine administration, the tendency was for the results in practically all types of tests to be normal within approximately two months.

SUMMARY

The findings obtained in the present study may be summarized as follows:

1. The results of the bromsulfalein test showed that:

(a) In 138 patients tested prior to fever therapy, fifteen per cent had abnormal values with only three individuals having 10 per cent or more retention.

(b) During fever therapy, of eighteen patients studied, fifteen, or 83 per cent, had abnormal values with ten ranging between 12 and 40 per cent. Following fever therapy fifteen of these patients had normal values.

(c) Following fever therapy sixty-six of the 138 patients were re-examined, and fifty-eight of them, or 88 per cent, were normal.

2. Cephalin flocculation tests using Wilson and Difco antigens showed that before inoculation and up until the first positive smear the percentage of 3 and 4 plus tests was zero; however, by the twenty-first day it was 100. No tests were performed after termination of fever.

3. Only one of 106 patients on whom galactose tolerance tests were performed prior to malarial therapy had an abnormal value. Following therapy thirty-one of these men were retested, and all had normal values.

4. The intravenous hippuric acid test was performed on twenty-five patients prior to fever therapy, and all results were normal. Twenty-two of these men were retested during fever therapy, and three had abnormal values. On the re-examination of two of them following therapy, one still remained abnormal. An additional ten of the original men were retested following fever therapy and gave normal findings.

5. The icterus index and serum bilirubin concentration were determined on the same sera of thirty-two patients. During the last seven days of fever therapy abnormal values were found in 38 and 17 per cent, respectively, with a rise to 47 and 24 per cent during the first four days of quinacrine treatment and a fall to 15 and 12 per cent in the next three days.

6. The urine urobilinogen test was performed on the same thirty-two patients just mentioned. All tests were normal prior to the first positive smear, but by seven days later 41 per cent were abnormal with a fall to 6 per cent abnormal tests at the end of quinacrine treatment.

CONCLUSIONS

1. Tests of liver function were carried out before, during, and after fever therapy in certain of 138 patients with neurosyphilis in whom malaria of Pacific or Mediterranean origin was induced by sporozoite or blood inoculation.

2. Although the results gave definite evidence of impairment of function in the majority of patients during fever therapy, serial studies during the two months following termination of fever with quinacrine showed a gradual return to normal, or to the condition prior to fever therapy, in practically all instances.

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STUDIES OF PANCREATIC FUNCTION

III. The Effect of Ligation of the Pancreatic Ducts Upon the Amylase and Lipidase Content of the Blood

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IT IS well known and agreed that the effect of ligation of the pancreatic ducts is an immediate rise in the serum amylase and lipidase values.[1-4] There is less agreement, however, in the matter of the more chronic effects of ligation of the pancreatic ducts upon the concentration of these enzymes in the blood. Gould and Carlson[1] reported transient rises in the blood amylase for a period up to six weeks following pancreatic duct ligation. Golden and associates[2] demonstrated a permanent increase in blood amylase in three out of four dogs following the same operation. This is difficult to explain in view of their finding that the pancreas in all four dogs was converted into small, thin, cordlike remnants. Popper and Sorter[3] kept two dogs alive for twelve months following pancreatic duct ligation. After a transient rise in the amylase at three weeks and six weeks postoperatively in one of the two animals, with a corresponding rise in the lipidase at three weeks, they found the amylase and lipidase values to be completely within the normal range for both dogs until the termination of the twelve months' period. Gross examination of the pancreas in both dogs showed it to be a thin, small, cordlike mass, while microscopic examination revealed an abundance of connective tissue with intact islets of Langerhans and small nests of cells which resembled acinar tissue. McCaughan[5] approached this problem differently by introducing a rubber balloon into the duodenum and distending it with water until the pressure reached 90 mm. Hg; oxalated blood samples then showed a rise in amylase of 250 per cent in one animal and 380 per cent in another. However, after ligating the pancreatic ducts and allowing time for the pancreas to atrophy, repetition of occlusion of the pancreatic ducts by the distended balloon caused no rise in the blood amylase.

A somewhat similar situation occasionally occurs in patients following an attack of acute pancreatitis. Pinkham[6] reported six cases in which there was persistent elevation of the serum amylase following the signs and symptoms of pancreatitis. Surgical exploration revealed the presence of collections of pancreatic fluid called pseudocysts, and drainage of these fluid collections promptly caused a decrease in the serum amylase in four of the six cases. Curiously enough, there was no quantitative relationship between the serum and the cyst fluid amylase values.

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This paper is a report of work designed to obtain information upon the relationship of blood amylase and lipidase to the atrophied, fibrotic pancreas. In another paper,[4] it was demonstrated that following complete pancreatectomy in cats there was an initial decrease in both the serum amylase and lipidase with a later irregular, but definite, return to control levels. A comparison of these results with the blood enzyme values in an animal with an atrophic pancreas, obtained by the same methods of analysis, was thought to be of interest.

EXPERIMENTAL

Four cats were anesthetized by intraperitoneal injection of nembutal, 30 mg. per kilogram of body weight. Control blood samples were obtained by heart puncture. After performing an upper midline incision, the pancreas was exposed and freed from the duodenum by blunt dissection. The area of the head of the pancreas containing the pancreatic ducts was doubly ligated with nonabsorbable sutures close to the ampulla of Vater and then cut between the ligatures. All pancreatic tissue remaining attached to the duodenum was carefully removed. The cut end of the pancreas was ligated again, and it was made certain that the pancreas was completely free of the intestine. The abdomen was closed with sutures and skin clamps. The animals were kept on a diet of dehydrated cat food, meat scraps from the hospital kitchen, and supplementary whole milk.

The determinations of serum amylase were carried out by the method of Somogyi,[7] while serum lipidase values were determined according to the method of Goldstein and Roe[8] (tributyrin substrate).

RESULTS

The results are expressed graphically in Table I.

A description of the gross autopsy findings and the microscopic histologic features follows. All tissues were stained with Harris' hematoxylin and eosin and Mallory's triple connective tissue stains.

TABLE I

PERIOD AFTER LIGATION (MO.)	UNITS PER 100 C.C. OF SERUM							
	CAT 1		CAT 2		CAT 3		CAT 4	
	AMYLASE	LIPIDASE	AMYLASE	LIPIDASE	AMYLASE	LIPIDASE	AMYLASE	LIPIDASE
Control	1,767	130	590	137	910	142	925	131
1	701	112	330	59	756	106	34	144
2	840	128	157	128	810	109	953	117
3	1,109	104	635	78	889	83	908	90
4	822	110	928	119	888	109	950	144
5	772	82	759	135	698	92	1,495	92
8	1,891	148	1,155	178	1,153	149	907	147
Appearance of pancreas								
Gross	Cystic		Cystic		Atrophic		Atrophic	
Microscopic	Normal areas		Incompletely atrophic		Incompletely atrophic		Completely atrophic	

Cat 1.—

Gross Findings: The pancreas appeared of normal length but was stringy and cordlike. No communication between the pancreas and the duodenum could be observed. A small pea-sized cyst filled with clear fluid was present on the exterior of the duodenum at the site of ligature, but no pancreatic tissue could be seen. The liver was of normal size but appeared yellowish and fatty.

Microscopic Examination: The pancreas was normal except for some eosinophilia of the distal cytoplasm of the acinar cells and some slight desquamation of the epithelium of a few of the alveoli. The islet and ductile tissue were normal. The liver showed small areas of intracellular fat infiltration and some abnormal dilation of the sinusoids.

Cat 2.—

Gross Findings: The pancreas appeared to be completely replaced by stringy, cordlike, fibrotic tissue. In the head of the pancreas near the second part of the duodenum a small hard mass which tapered off into the replaced pancreas could be palpated. On cutting into it, it was seen to be cystic, containing a clear watery fluid and continuous with the main pancreatic duct. No communications between the pancreas and duodenum could be demonstrated.

Microscopic Examination: The pancreas showed a marked increase in the interlobular connective tissue and a considerable reduction of acinar tissue. This was confined to small encapsulated areas among the extensive "scar" tissue. Most of the residual alveoli had dilated lumens lined with flattened epithelium. Rather extensive aggregations of lymphocytes had infiltrated into the hypertrophied connective tissue. Histiocytes and fibroblasts were numerous in these small round-cell clusters. There was no evidence of fat necrosis. The islet tissue was normal. The liver was normal except for an unusual number of von Kupffer cells.

Cat 3.—

Gross Findings: The pancreas appeared stringy and cordlike but of normal length, extending to the spleen. A small cyst, pinhead in size, was present at the site of ligature at the head of the pancreas.

Microscopic Examination: The pancreas had only a small amount of normal acinar tissue. Most of the alveoli showed a considerable amount of desquamation of the secretory epithelium. The lumens of some acini were dilated and the epithelium was flattened. The interlobular connective tissue showed slight hyperplasia. The islet and ductile tissues were normal. The liver was normal.

Cat 4.—

Gross Findings: The remnants of the pancreas were extremely difficult to recognize and identify as pancreas, since the area was almost completely fibrotic. A small cyst was present at the site of ligature at the head of the pancreas. No communication between the remnants of the pancreas and the duodenum could be found.

Microscopic Examination: The pancreas showed complete atrophy of the acinar tissue. Small nests of normal islet tissue were found in the markedly increased connective tissue septa. Some areas of lymphocytic infiltration were noted. A large dilated duct occupied a sizable portion of the atrophic gland. The liver was normal.

DISCUSSION

In a previous work[4] we have demonstrated an immediate rise in serum amylase and lipidase following pancreatic duct ligation. It was the purpose of these experiments to show later effects of ligation of the pancreatic ducts. At the one- and two-month intervals after ligation, all four animals showed amylase concentrations below the control values, and, in some instances, the values were remarkably subnormal. Following this, there was a gradual rise in amylase concentrations until the eighth month after ligation, when the amylase determinations had returned to the preoperative levels.

The lipidase determinations were less clear-cut, but in the first three months postoperatively there was a recognizable decrease in the serum lipidase values. Following this period, there was a gradual rise in the lipidase concentrations until the eighth month after ligation, when the lipidase values were essentially the same as the control values.

It is of considerable interest that in the microscopic examination one pancreas showed normal areas of acinar tissue, two showed considerable necrosis with little recognizable acinar tissue, and the fourth was completely atrophic. In spite of the variations in the microscopic picture, the serum amylase and lipidase of all four animals show the same postoperative trends. The obvious conclusion is that subsequent return to control levels was due to extra pancreatic factors.

If the conclusions from these laboratory experiments can be translated to clinical laboratory findings, it follows that the serum lipidase determination is as nonspecific as the amylase test in chronic pancreatic disease.

These data support the same conclusions arrived at by total pancreatectomy experiments, namely, that there are other sources of amylase and lipidase in the animal body which may operate to produce as high a level of these enzymes in the blood as when a normally functioning pancreas is present.

SUMMARY

1. Studies of the amylase and lipidase content of the blood serum of cats following ligation of the pancreatic ducts over a period of eight months have been made.

2. Serum amylase and lipidase concentrations, which previous work has shown are at first rapidly increased by ligation of the pancreatic ducts, showed marked decreases in from one to three months after ligation.

3. In one animal with a completely atrophic pancreas and in three others with varying degrees of pancreatic necrosis due to ligation of the pancreatic

ducts, the serum amylase and lipidase concentrations returned to control levels in eight months postoperatively.

4. These data appear to show that the amylase and lipidase concentrations of the blood serum are markedly influenced by extra pancreatic factors.

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THE PRODUCTION AND COURSE OF ALLOXAN DIABETES IN THE RAT

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IT WAS usually noted in preliminary studies on the rat that when alloxan alone was given in doses of 200 mg. per kilogram more than 80 per cent of the animals developed diabetes (Lazarow, 1945[1]). Occasionally, however, a group of animals failed to respond to this dose of alloxan. Because it is desirable to be able to produce diabetes consistently in the rat, the present study was undertaken.

Numerous investigators have studied alloxan diabetes in the rat. Although all have used parenteral routes, there is disagreement as to the best method of producing diabetes. Dunn and McLetchie (1943)[2] used repeated subcutaneous injections. Gomori and Goldner (1943)[3] reported consistent results using one intraperitoneal injection of alloxan (200 mg. per kilogram) and stated that subcutaneous injections of 50 to 175 mg. per kilogram were not effective. Bailey, Bailey, and Leech (1944),[4] however, reported that intraperitoneal and intravenous injections of alloxan in the rat were not satisfactory, whereas a subcutaneous dose of 200 mg. per kilogram did produce diabetes regularly. Gomori and Goldner also reported that hooded rats were resistant to alloxan. But Duff and Star (1944)[5] stated that if hooded rats are injected with larger doses (from 175 to 350 mg. per kilogram) of alloxan they, too, develop diabetes.

Since alloxan is rapidly destroyed in body fluids (or in the blood), it seems that theoretically, at least, the intravenous route of administration would be preferable. This is actually the case, for the intravenous method avoids the highly variable factor of destruction which occurs during alloxan absorption, and hence, diabetes is produced more consistently. The amount of alloxan needed to produce a given level of diabetes is but a small fraction of that required when the alloxan is administered by a nonintravenous route. This paper compares the diabetes produced in the rat by alloxan administered intravenously with that produced by intraperitoneal injection.

METHODS

Sprague-Dawley male and female albino rats were used in this study and were allowed excess food and water. For the intravenous injection rats were immobilized by placing them in a cylindric mailing carton of appropriate size. This carton was provided with air holes at one end and at the other end with a

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rubber stopper having a cutout through which the animal's tail protruded. With the rat inside, this carton was placed on the heating coil of a 56° C. incubator and was heated for a minute or longer in order to induce dilatation of the tail veins. When the heating was adequate, the tail veins were distinctly visible. When slight pressure was applied with a finger over the base of the tail, the distal portion of the vein became distended with blood and could be easily tapped with a No. 26 hypodermic needle. One should be able to draw blood into the syringe both before and after the completion of the injection. Once the technique of intravenous injection is mastered, rats can be injected at the rate of thirty per hour.

Alloxan monohydrate was prepared as a 2 to 5 per cent aqueous solution and was injected in various doses from 15 to 200 mg. per kilogram intravenously and from 50 to 200 mg. per kilogram intraperitoneally. Serial blood sugars were determined by the Folin-Malmros micro method (1929)[6] at 0, 3, 8, 24, 48, and, at times, 72 hours. Nonprotein nitrogen determinations were carried out on the tungstate filtrates obtained in the Folin-Malmros procedure. The filtrates were charred and microdistilled, and the nitrogen was determined by direct nesslerization, using Koch's modification (1941)[7] of the Nessler-Folin reagent. One-tenth of a cubic centimeter of blood obtained by cutting the tip of the tail sufficed for both the blood sugar and the N.P.N. determinations. Following the last sugar determination the animals were killed by a blow on the head, perfused through the aorta with a small amount of saline, followed by about 200 c.c. of formol-Zenker solution. Tissues were immersed from six to ten hours in formol-Zenker solution; then they were washed, dehydrated, and embedded in paraffin according to the usual methods. Sections of the pancreas, kidney, liver, and adrenal were stained with hematoxylin-eosin. The pancreas was also stained by a slight modification of Bensley's neutral crystal violet method (Palay and Lazarow, 1946[8]). Selected sections of liver were stained by Wilder's stain for reticulum.

RESULTS

Blood Sugar Response.—Following the injection of alloxan three phases in the blood sugar response were noted: an initial hyperglycemia, followed by a transient hypoglycemia, and finally, a late stage of permanent hyperglycemia or diabetes. The durations of these phases were somewhat variable from animal to animal. The blood sugar values of normal rats allowed excess food and water practically always fell within the range of 90 to 160 mg. per 100 c.c.

In Fig. 1 is shown the blood sugar response of thirty-six rats given alloxan intravenously in doses of 40 mg. per kilogram. The blood sugar value is designated by the height of the rectangle. At three hours only hyperglycemic reactions were noted. Hypoglycemic reactions appeared at twenty-four hours and a few at forty-eight hours. Many hypoglycemic reactions were probably missed because of the limited number of blood sugar determinations. By forty-eight hours, however, most of the animals had reached their permanent hyperglycemic phase or diabetes.

Two of the thirty-six animals receiving 40 mg. per kilogram of alloxan showed a hypoglycemic reaction at forty-eight hours. Two others which had normal sugars at forty-eight hours had apparently just passed through the hypoglycemic phase and probably would have developed hyperglycemia at seventy-two hours had they been permitted to survive. Animals whose blood sugars at no time exceeded 160 mg. per cent or fell below 90 mg. per cent were

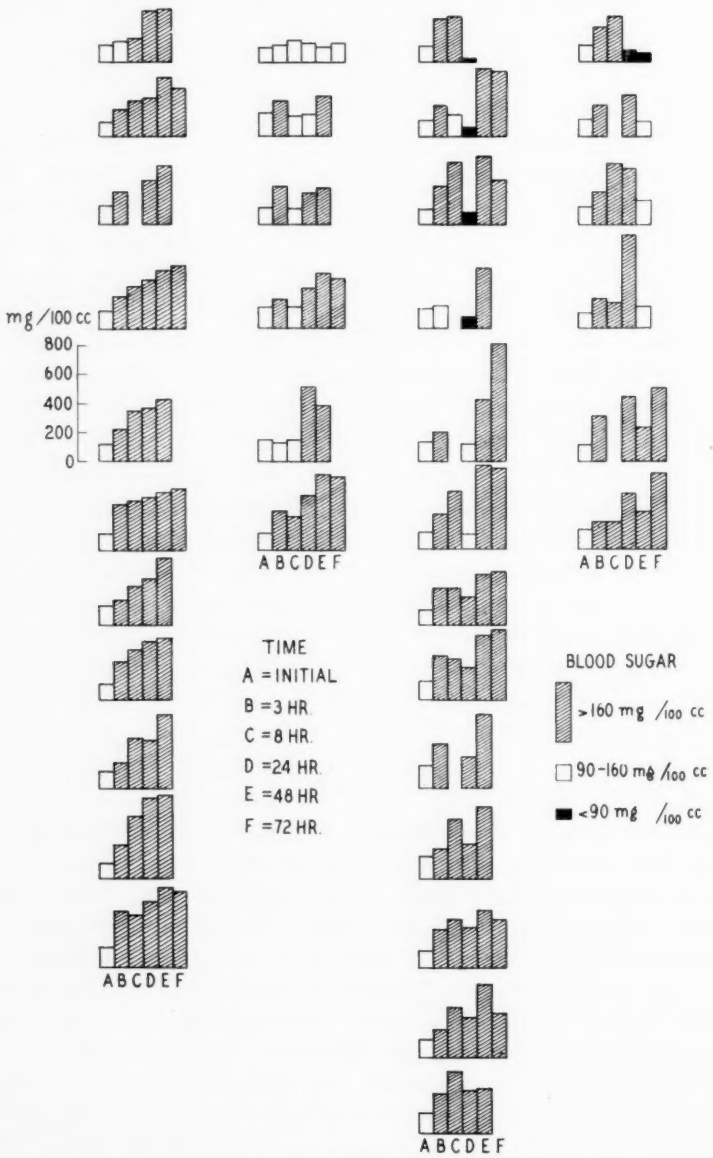

Fig. 1.—Blood sugar response of thirty-six rats given 40 mg. per kilogram of alloxan intravenously. Serial blood sugars were determined at 0, 3, 8, 24, 48, and, occasionally, 72 hours. The blood sugar value is given by the height of the rectangle.

considered as showing no response to alloxan. As a general rule the level of the blood sugar at forty-eight hours was a measure of the severity of the diabetes.

Relation of Dose of Alloxan to Blood Sugar Response.—The incidence of blood sugar responses as well as the average forty-eight hour blood sugar in each group is given in Table I. The forty-eight hour average includes the blood sugars of those animals which did not show any response to alloxan.

TABLE I. COMPARISON OF EFFECTS OF GRADED DOSES OF ALLOXAN INJECTED INTRAVENOUSLY AND INTRAPERITONEALLY

DOSE ALLOXAN (MG. PER KG.)	TOTAL NUMBER OF ANIMALS	AVERAGE INITIAL WEIGHT (GM.)	PER CENT WEIGHT LOSS (AT 48 HOURS)	PER CENT ANIMALS WITH BLOOD SUGAR RESPONSE	AVERAGE 48-HOUR BLOOD SUGAR	PANCREATIC DAMAGE (NUMBER OF ANIMALS)				BLOOD N.P.N. (NUMBER OF ANIMALS)					KIDNEY TUBULE NECROSIS (NUMBER OF ANIMALS)				LIVER NECROSIS (NUMBER OF ANIMALS)			
						SEVERE	MODERATE	MINIMAL	NORMAL	AVERAGE (AT 48 HOURS)	INCREASE (50% OR LESS)	INCREASE (50 TO 150%)	INCREASE (150 to 350%)	INCREASE (350% OR MORE)	NORMAL	SLIGHT	MODERATE	SEVERE	NORMAL	SLIGHT	MODERATE	SEVERE
Intravenous																						
100	2	150	13	100	–	2	0	0	0	508	0	0	0	2	0	0	0	2	2	0	0	0
75	2	171	15	100	933	2	0	0	0	223	1	0	0	1	2	0	0	0	1	1	0	0
50	8	186	10	100	509	8	0	0	0	182	4	2	0	2	3	4	1	0	6	0	0	2
40	18	204	10	94	333	17	1	0	0	42	17	0	1	0	16	2	0	0	6	4	3	5
30	4	192	3	75	346	2	2	0	0	67	3	1	0	0	3	1	0	0	2	0	0	2
25	2	230	5	50	239	1	1	0	0	46	2	0	0	0	2	0	0	0	2	0	0	0
20	4	146	0	25	189	1	1	0	2	47	4	0	0	0	3	1	0	0	2	0	1	1
15	2	220	0	0	126	0	1	0	1	41	2	0	0	0	2	0	0	0	2	0	0	0
Intraperitoneal																						
200	8	241	12	75	366	6	2	0	0	133	1	3	1	3	0	3	2	3	3	4	0	1
150	3	307	6	66	396	2	0	0	1	66	1	2	0	0	1	2	0	0	0	2	1	0
100	4	184	3	0	116	0	1	2	1	52	4	0	0	0	3	1	0	0	3	1	0	0
50	4	159	0	0	109	0	0	1	3	39	4	0	0	0	4	0	0	0	3	1	0	0

It is clear that when alloxan was administered intravenously in doses of 50 mg. per kilogram or greater all of the rats responded. After a dose of 40 mg. per kilogram intravenously seventeen of eighteen rats responded. In sixteen additional rats given 40 mg. per kilogram of alloxan intravenously and not included in Table I, all showed a blood sugar response. As the alloxan dose was reduced, the incidence of blood sugar response decreased, and at a dose of 15 mg. per kilogram intravenously none showed any blood sugar response. In contrast to these results following intravenous administration, when 200 mg. per kilogram were given intraperitoneally, only six of eight animals showed a blood sugar response, and after a dose of 100 mg. per kilogram intraperitoneally, none showed any blood sugar response. Thus 40 mg. per kilogram of alloxan administered intravenously produced a more consistent effect than did 200 mg. per kilogram administered intraperitoneally.

The severity of the blood sugar response is directly related to the dosage of alloxan administered. Thus, after doses of 75 to 200 mg. per kilogram intravenously, many of the forty-eight hour blood sugar values were in the neighborhood of 1,000 mg. per cent. As the alloxan dose was reduced, the blood sugar at forty-eight hours was also less elevated. At 40 mg. per kilogram intravenously most of the forty-eight hour blood sugars fell between 200 and 500 mg. per cent, and in only one animal out of thirty-four was it over 600 mg. per cent. At still lower dosages those animals which reacted to alloxan usually showed a forty-eight hour blood sugar of 300 to 400 mg. per cent. The average forty-eight hour blood sugar value, however, given in Table I is weighted by the inclusion of the animals that did not react.

When doses of 200 mg. per kilogram of alloxan were injected intraperitoneally, the average forty-eight hour blood sugar was about the same as that observed following an intravenous dose of 40 mg. per kilogram. Although the group of rats which received 200 mg. per kilogram intraperitoneally was smaller than the group which received 40 mg. per kilogram intravenously, the distribution of the forty-eight hour blood sugars of the diabetic animals in the two groups was the same. Thus, the severity of the diabetes produced by alloxan injected intravenously in doses of 40 mg. per kilogram was of about the same degree as that produced by the intraperitoneal injection of alloxan in doses of 200 mg. per kilogram.

Weight Loss.—Animals which showed a blood sugar response following the injection of alloxan also showed considerable weight loss in forty-eight hours, usually about 10 per cent of their body weight.

Pancreatic Damage.—The morphologic changes produced by alloxan in the islets of Langerhans have recently been reviewed by Duff (1945).[9] These changes consist mainly of selective necrosis and disappearance of the beta cells with consequent destruction of the architecture of the islets. The beta cells undergo degranulation, shrinkage, and nuclear pyknosis and are finally removed from the islet. The completely affected islet typically is composed of a cluster of capillaries surrounded by a band of normal alpha cells. The absence of inflammatory reaction is striking.

In Table I the degree of damage to the islets has been classified into three main groups: severe, moderate, and minimal.

In islets which were included in the first group, the beta cells had either completely disappeared or were all shrunken and detached and contained pyknotic nuclei. Islets which contained necrotic beta cells and a moderate number of normal beta cells were considered as moderately damaged. Included in the same group were islets in which most of the beta cells were simply degranulated but still contained normal nuclei. When the architecture of the islets was intact and most of the beta cells were normal (a few necrotic or degranulated), the islets were considered to show only minimal changes.

In general, there was very good correlation between the histologic findings and the blood sugar response (Table I). When the pancreatic damage was

severe, all animals showed the typical blood sugar response and developed diabetes. However, the severity of the diabetes as judged by the forty-eight hour blood sugar could not be predicted by studying the extent of cytologic damage in the pancreas. Foglia (1944)[10] observed that after removal of 95 per cent of the pancreas rats developed manifest diabetes only after several months. The progression of the disease in such partially pancreatectomized animals may be attributed to degeneration of the remaining beta cells (Allen, 1913[11]; Homans, 1913, 1914[12]). Therefore, it is reasonable to suppose that in order to produce immediate diabetes in the rat by alloxan, it is probably necessary to destroy or inactivate more than 95 per cent of the beta cells. Probably one cannot expect to recognize by histologic examination slight increments in the extent of beta cell damage beyond 95 per cent destruction. As the dose of alloxan was reduced, however, many of the animals which showed moderate changes in the pancreas (that is, degranulation or necrosis of only some of the beta cells) did not develop diabetes and showed only very slight, if any, fluctuations in their blood sugars. Thus, cytologic examination of the pancreas will detect moderate damage of the beta cells more accurately than will a study of the blood sugar response.

Renal Damage.—In addition to the pancreatic lesions, alloxan also produces renal damage. The tubules are usually involved, and the severity of the injury varies from hydropic degeneration to necrosis (Bailey, Bailey, and Leech, 1944[4]; Dunn and McLetchie, 1943[2]). In our series necrosis of the tubules was commonly focal, but when extensive it involved all lobules of the kidney. The affected tubules usually were filled with necrotic cells and debris. Often all the epithelial cells had desquamated into the lumen. In some cases, however, there were many young epithelial cells containing basophil cytoplasm and nuclei in mitosis, an indication that repair had begun. Infiltration of polymorphonuclear leucocytes around the affected tubules occurred in only a few specimens.

In Table I the extent of tubular necrosis is listed. A kidney showing only occasional isolated small foci of tubular necrosis was considered to be slightly damaged. A kidney with many isolated necrotic areas was considered to be moderately damaged, and one in which the areas of necrosis were large and confluent was considered to be severely damaged. Hydropic degeneration and cloudy swelling are not listed in the accompanying table, because the extent of this type of damage was more difficult to classify than was necrosis.

Both the incidence and severity of the renal lesions were directly related to the dose of alloxan administered. At 40 mg. per kilogram intravenously sixteen of eighteen rats showed no significant changes, whereas two showed only slight changes in the kidney tubules. In contrast, at 200 per kilogram intraperitoneally, none of the rats were entirely normal, and more than half of the animals showed moderate to severe changes in the kidney. Thus, although the intravenous administration of 40 mg. per kilogram of alloxan was as effective in producing diabetes as was the larger intraperitoneal dose of 200 mg. per kilogram, the incidence of lesions in the kidney was markedly reduced by giving the smaller dose intravenously.

Blood Nonprotein Nitrogen.—In Table I is listed the average forty-eight hour N.P.N. and also the degree of elevation in N.P.N. for the various animals within the group. Fluctuations with ±50 per cent of the initial N.P.N. were considered within the normal range, for the animals were not fasted and control animals sometimes showed this much fluctuation. Again, when the effect of 40 mg. per kilogram intravenously is compared with that produced by 200 mg. per kilogram intraperitoneally, it is noted that seventeen of eighteen animals given the lower dose intravenously showed no significant elevation in the N.P.N. Only one animal showed an increase in its N.P.N. value of 200 per cent. In contrast, however, after the larger dose intraperitoneally, four of eight animals showed an increase of 300 per cent or greater, and three showed an increase in N.P.N. of 50 to 150 per cent. Only one of the eight animals in this latter group showed no significant change.

In general, the elevation in the nonprotein nitrogen of the blood following alloxan injection paralleled the severity of the renal damage observed histologically. In a few instances there was evidence of slight kidney necrosis without any significant rise in the N.P.N. In three cases which had received 40, 50, or 75 mg. per kilogram of alloxan intravenously, the forty-eight hour N.P.N. was markedly increased, from 113 to 399 mg. per cent, and yet the kidney exhibited only slight evidence of necrosis. The tubules, however, did show considerable hydropic degeneration. Two of these animals were also unusual in that they passed through a delayed hypoglycemic phase at forty-eight hours, and all showed a very intense hyperglycemia at some time during the forty-eight hours, from 629 to 1244 mg. per cent. How much of the azotemia was due to renal and how much to extrarenal factors is difficult to determine. However, since uric acid values up to 100 mg. per cent have been reported in the pigeon following alloxan administration (Goldner and Gomori, 1945[13]), part of the elevation in N.P.N. might be due to this nitrogenous substance.

Liver Damage.—The typical lesion produced in the liver by intravenous injection of alloxan was a small circular focus of necrosis usually located in the midzonal or peripheral region of the lobule. The lesions, which varied in diameter from 45 to 200 micra, were usually smaller than 100 micra and were distributed uniformly throughout all lobes of the liver. Intact liver cells were usually completely absent from the focus, and the sinusoids were collapsed or distorted. The normal reticular framework was fragmented or absent. The center of the lesion was occupied by large mononuclear cells which appear similar to the Kupffer cells in the surrounding intact parenchyma. These cells frequently contained fragments of pyknotic nuclei or hyalin acidophil masses. Inflammatory reaction was usually slight or absent. Shrunken acidophil remnants of liver cells were scattered throughout the lesion and at the margins. The liver cells in the surrounding cords were either entirely normal or contained large cytoplasmic vacuoles and hyperchromatic nuclei. When the liver showed extensive necrosis, the remaining parenchyma usually showed fatty metamorphosis; however, in livers with only occasional foci of necrosis, the rest of the organ was usually normal.

In Table I the necrosis of the liver following intravenous injection of alloxan has been classified according to the extent of involved tissue. When only occasional foci of necrosis appeared in the cross sections of the entire liver, the damage was considered slight. In moderately affected livers there was approximately one focus of necrosis in the area of three or four lobules. Severely affected livers had at least one focus of necrosis in each lobule.

When alloxan was given intraperitoneally, the necrosis, although focal, was not dispersed throughout the organ. The lesions were principally subcapsular and limited to the free edge of the lobes, and the subdiaphragmatic portions of the liver suffered little or no damage. The necrosis often involved a band only two or three cells deep under the peritoneum, but in a few specimens the necrosis extended deep into the liver substance. In only one rat was the damage sufficient to be called severe.

The incidence and severity of the hepatic involvement cannot be adequately correlated with either the dosage of alloxan or the incidence of renal lesions. Some of the highest intravenous alloxan doses did not produce hepatic lesions, whereas some of the lowest were associated with severe damage. In some of the animals which showed extensive renal involvement, the livers were normal; whereas in others severe hepatic necrosis was not associated with renal change. Very occasionally after administration of a nondiabetogenic dose of alloxan, some hepatic involvement was noted in the absence of any pancreatic lesions.

Course of Diabetes Produced by Injection of 40 Mg. Per Kilogram of Alloxan Intravenously.—Sixteen rats were given alloxan in doses of 40 mg. per kilogram intravenously and were followed serially for several months (Table II). They were kept on a diet of Friskies dog food* and water. The blood sugars, weights, and water intakes were determined daily. All sixteen rats responded to alloxan.

TABLE II. ALLOXAN DIABETES IN THE RAT (40 MG. PER KILOGRAM INJECTED INTRAVENOUSLY)

RAT	INITIAL WEIGHT	DAYS SURVIVAL	AVERAGE BLOOD SUGAR VALUE						
			FIRST WEEK	SECOND WEEK	THIRD WEEK	FOURTH WEEK	FIFTH WEEK	SIXTH WEEK	SEVENTH WEEK
8	278	Alive	291	136	123	132	122	132	134
6	274	Alive	339	191	247	286	382	369	402
5	287	Alive	353	258	322	396	378	366	389
3	293	Alive	378	409	426	438	450	430	416
13	288	Alive	337	332	408	404	458	450	498
14	317	Alive	368	406	429	482	486	466	491
1	258	Alive	444	417	430	599	565	505	544
11	330	Alive	352	403	434	506	516	564	540
15	298	29	409	436	488	542	–	–	–
2	292	23	456	451	582	704	–	–	–
7	264	4	355	–	–	–	–	–	–
4	228	4	504	–	–	–	–	–	–
16	290	4	360	–	–	–	–	–	–
17	284	4	578	–	–	–	–	–	–
18	299	5	358	–	–	–	–	–	–
12	309	6	406	–	–	–	–	–	–

*Albers Milling Co.

Five animals died between the fourth and sixth days with terminal blood sugars ranging from 360 to 560 mg. per cent. One of the animals showed a significant increase in the blood N.P.N. at forty-eight to seventy-two hours; the others were normal at this time. A sixth animal (Rat 16), although it had a blood sugar of 475 mg. per cent at forty-eight hours, died on the fourth day with a blood sugar of only 190 mg. per cent just prior to death. All of the remaining ten rats survived many weeks. Of the surviving animals one (Rat 8) showed transitory diabetes lasting for five or six days (Fig. 2). After this period the blood sugar returned to normal, the weight started to increase, and the water intake fell off to normal. A second animal (Rat 6), whose blood sugar at forty-eight hours was 530 mg. per cent, showed wide fluctuations in the daily blood sugar which frequently fell below 200 mg. per cent. However, as time progressed the average blood sugar tended to rise, fluctuations below 200 mg. per cent occurred less frequently, and finally, after thirty-six days the blood sugar tended to stabilize at about 370 to 400 mg. per cent. During the period of progressive rise in blood sugar the water intake increased, and the weight gain was less rapid than that occurring in the early stages of the diabetes.

During the first several weeks following the onset of the diabetes most of the eight remaining rats showed a slight but progressive increase in their average blood sugars (Table II). For example, the average blood sugar of Rat 15 increased from 409 mg. per cent during the first week to 436 the second, 488 the third, and 542 the fourth. Rat 2 which had an average sugar of 456 during the first week died on the twenty-third day with a terminal sugar of 824 mg. per cent.

In Fig. 2 is shown the course of the diabetes for selected animals from this group. The weight curves of these animals depended upon the severity of the diabetes. When the blood sugar value was moderately elevated, the weight tended to be maintained (Rat 6). With higher blood sugar values the rats tended to lose weight progressively (Rats 2 and 3). Some of these diabetic rats drank more than their own weight of water within twenty-four hours.

DISCUSSION

It is clear from these studies that the intravenous administration of alloxan in doses of 40 mg. per kilogram is a satisfactory method for the production of diabetes in the rat. Alloxan is an unstable compound which is destroyed rapidly at pH 7.4. It has been reported that it disappears from the blood within two minutes after its injection (Leech and Bailey, 1945[14]). Consequently it is not surprising that the dose of alloxan required to produce diabetes by the intraperitoneal route is much larger than that required by the intravenous route. Variations of the absorption rates of alloxan coupled with its rapid destruction during absorption could account for the inconsistencies reported in the literature. The intravenous method, however, eliminates these variable factors. It should be emphasized that the alloxan must be injected as rapidly as possible because during slow injection there may be increased destruction. Lower doses for intravenous injections have been used in the dog (Goldner and Gomori, 1943[15]).

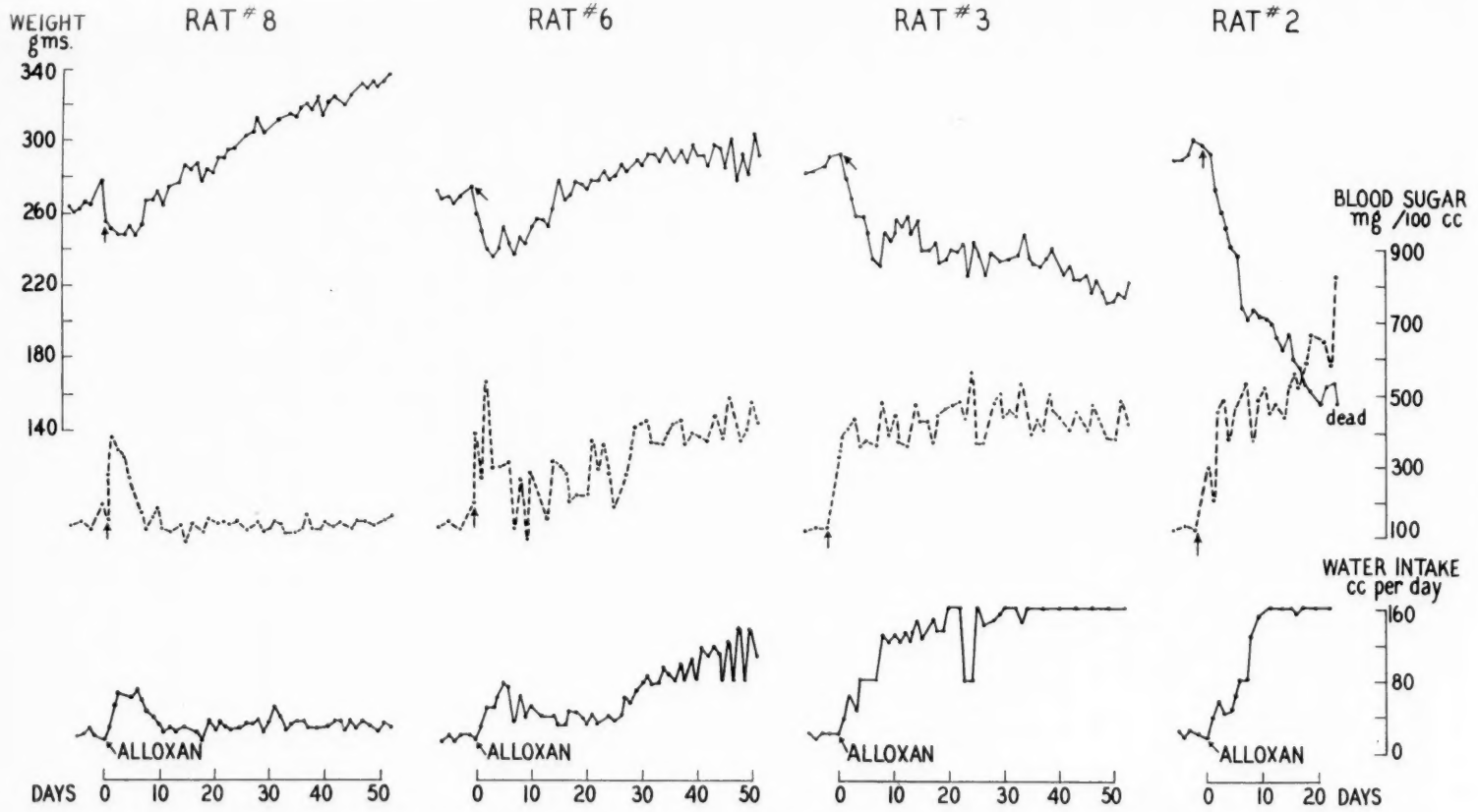

Fig. 2.—Course of alloxan diabetes in the rat (four selected cases). Alloxan was given in doses of 40 mg. per kilogram intravenously. The weights, blood sugars, and water intakes were measured daily.

It is interesting that some animals given 40 mg. per kilogram of alloxan intravenously showed a progressive increase in the severity of their diabetes as judged by the average blood sugar. After partial pancreatectomy rats may show a period of obesity without diabetes, followed by a period of latent diabetes, and finally, a period of manifest diabetes (Foglia, 1944[10]). Thus the beta cells remaining, although adequate to meet the body's needs for a time, later are unable to secrete sufficient amounts of insulin, and hence, the animal develops diabetes. Overwork and exhaustion of the beta cells have been given as explanations for this progression (Allen, 1913[11]; Homans, 1913, 1914[12]). Following a dose of alloxan of 40 mg. per kilogram most of the beta cells are destroyed. But it is likely that a few functional beta cells still remain, for when larger doses of alloxan are given intravenously (that is, from 75 to 100 mg. per kilogram), the diabetes which develops within forty-eight hours is more severe. Therefore, the progressive rise in the blood sugar observed over a period of weeks in rats given only 40 mg. per kilogram of alloxan may result from the exhaustion of the few remaining beta cells.

SUMMARY

1. The rapid intravenous injection of alloxan is more satisfactory for producing diabetes in the rat than is intraperitoneal injection.

(a) When alloxan is injected intravenously only a small dose is required, and the incidence and severity of the resulting diabetes are more uniform than when five times that dose is given intraperitoneally.

(b) By the intravenous injection of 40 mg. per kilogram of alloxan it is possible to produce diabetes in most rats without significant renal damage.

2. Sixteen rats were given 40 mg. per kilogram of alloxan intravenously, and their blood sugars, weights, and water intakes were followed for many weeks.

(a) Of these sixteen rats five died within the first four to six days with terminal blood sugars ranging from 360 to 560 mg. per cent.

(b) Ten of the sixteen rats survived many weeks.

(c) One which developed transitory diabetes during the first week recovered and remained normal for the following six weeks.

(d) During the first few weeks following the onset of diabetes, the average blood sugar levels of most of the surviving rats showed a slight but progressive rise and usually attained values of 400 to 600 mg. per cent.

(e) Depending on the severity of the diabetes the animals gained weight very slowly or progressively lost weight.

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THE USE OF HEPARIN WHEN PERFORMING EXCHANGE BLOOD TRANSFUSIONS IN NEWBORN INFANTS

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AT LEAST three distinct clinical entities are grouped under the term erythroblastosis fetalis.[1-3] The importance of recognizing the differences between these entities is that the treatment and prognosis vary with the manifestation. Those cases that are characterized by anemia with slight jaundice (congenital hemolytic disease) respond readily to proper transfusion therapy, while infants who show severe jaundice with little or no anemia (icterus gravis) frequently die within a short time after the onset of the disease, usually with the postmortem finding of kernicterus.[4] Of this latter group some survive but may later develop sequelae of severe cerebral damage or hepatic cirrhosis.[5]

The fact that the disease usually has its onset at birth or very shortly thereafter offers the hope that if a reasonably accurate prediction can be made during pregnancy concerning the likelihood of the disease occurring, as well as the type of manifestation, the lives of these babies can be saved and the sequelae prevented. The newer tests for the Rh-Hr types, as well as the development of methods of detecting the presence or absence of sensitization, now offer the prospect of more accurate forecasting.[6, 7] If, as has been proposed by one of us, the syndrome of icterus gravis is initiated by intravascular agglutination or conglutination,[1-3] then exchange transfusion with blood completely compatible with the maternal serum should effectively prevent the onset of the disease process or arrest it after it has begun.

The purpose of this paper is to describe a technique of performing exchange transfusions in newborn infants, which has been successful in our hands.

THEORETIC CONSIDERATIONS

If the infant is to survive the procedure, blood must be injected and withdrawn simultaneously and at approximately equal rates.

Let us assume that in an infant with the blood volume V the speeds of withdrawal and injection of blood are uniform and equal. Let T represent the time required to withdraw and inject a quantity of blood equal to V and t the time to inject and withdraw any given volume of blood, while v represents the volume of donor's blood in the patient's circulation at time t. Then:

$$\text{When } t = 0,\ v = 0 \text{ and } \frac{dv}{dt} = \frac{V}{T}.$$

$$\text{When } t = \infty\ v = V \text{ and } \frac{dv}{dt} = 0$$

$$\text{Therefore, } \frac{dv}{dt} = \frac{V - v}{T}$$

$$\text{Hence, } v = V\ (1 - e^{-\frac{t}{T}})$$

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More simply, the proportion of foreign blood ($\frac{v}{V}$) is $1 - e^{-t}$ if t is expressed in units of T. In Fig. 1 this is shown in graphic form.

By substituting numeric values for t, the data in Table I are obtained.

For practical purposes the blood volume of a newborn infant can be considered to be about 250 c.c., so little is gained by the transfusion of more than 500 c.c. of blood. As Wallerstein[8] has pointed out, it is advantageous to inject from 50 to 100 c.c. more blood than has been withdrawn, which serves further to increase the proportion of donor's blood.

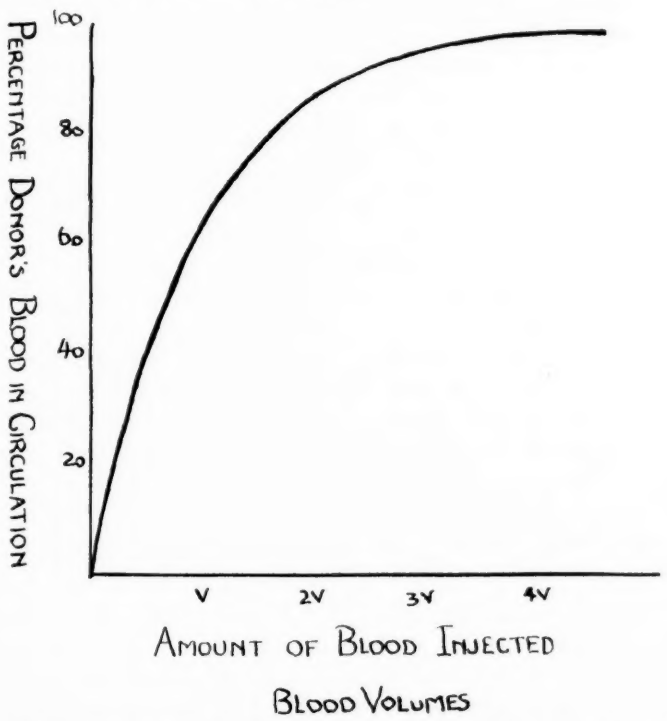

Fig. 1.—Percentage of donor's blood in patient's circulation after infusion and withdrawal of varying volumes of blood. In this figure, V represents an amount of blood equal to the patient's blood volume.

TECHNIQUE

The main obstacle to the successful performance of an exchange transfusion in infants is not the injection but the process of withdrawal, which is hampered by the coagulation of the infant's blood. This is circumvented by the use of heparin.

To test the efficacy of the procedure, a Mongolian idiot, 28 days old, weighing 7 pounds, was used. The infant belonged to group $A_2MNRh_1Rh_2$. A sample of bank blood drawn into sodium-citrate-citric-acid-dextrose mixture four days previously, belonging to group $A_1MRh_1Rh_2$ and compatible on cross-match, was selected for the transfusion. The cells had sedimented, and we removed about

TABLE I.

AMOUNT OF BLOOD INJECTED	PERCENTAGE OF DONOR BLOOD IN BODY
½ Blood volume	39.4
1 Blood volume	63.2
1½ Blood volumes	77.7
2 Blood volumes	86.5
2½ Blood volumes	91.8
3 Blood volumes	95.0

three fourths of the plasma and made the total volume up to 500 c.c. again with sterile saline solution. The internal saphenous vein was exposed at the left ankle, and a blunt 20 gauge cannula was tied in place with catgut. One-half cubic centimeter of Upjohn's heparin, equivalent to 500 units, was injected through the needle, and the gravity apparatus containing the blood was connected. There was considerable oozing at the site of the incision, but this was readily controlled by pressure. An incision was then made at the right wrist and a short bevel 20 gauge needle inserted into the radial artery. By this time the infant had received about 50 c.c. of blood through the drip infusion. The infant's blood flowed freely from the radial puncture and was collected fractionally for differential agglutination studies. The speed of the infusion was regulated constantly to keep pace with the radial flow and to maintain the original 50 c.c. advantage.

When 150 c.c. of blood had been withdrawn, some clotting occurred in the radial cannula; therefore, another 0.5 c.c. of heparin was administered, this time into the radial artery. Except for one short period when the infant became pale and showed evidence of air hunger, the procedure went quite smoothly. The infant's color and normal respirations were re-established by increasing the rate of flow of the infusion. After 400 c.c. of blood had been withdrawn, the radial artery was ligated and the skin sutured. The infusion was permitted to continue until the entire 500 c.c. of blood were administered. The saphenous vein was then ligated and the skin sutured. Only slight oozing occurred, and this was readily controlled by pressure bandages. The infant's condition at the end of the procedure was excellent. A blood count done on the following day showed a hemoglobin concentration of 88 per cent* and red cell count of 4.2 million per cubic millimeter. A blood count taken two weeks prior to the procedure showed a hemoglobin concentration of 120 per cent and red cell count of 4.7 million. The larger drop in hemoglobin concentration in comparison with the drop in the red cell count is due to the substitution of normocytic cells for the macrocytic cells which characterize the neonatal period.

The efficacy of the procedure was demonstrated by the differential agglutination test[9] done on the samples of blood withdrawn from the infant at various stages of the procedures. The increase in the proportion of donor's cells roughly followed the pattern shown in Fig. 1, and the last sample of blood showed that even before the final 50 c.c. had been injected approximately 90 per cent of the red cells in the infant's circulation were the donor's. Incidentally, the blood samples in all the tubes except the last failed to clot even

*One hundred per cent equivalent to 15 Gm. per 100 c.c.

when kept overnight. The occurrence of clotting in the last tube was probably due to the washing out of the heparin from the circulation by the infused blood. It appears that the amount of heparin used is innocuous, because in a previous test designed to determine a safe heparin dosage for the procedure, quantities as high as 5 c.c., or five times the amount used in the transfusion, were given intravenously without harmful effects, even though the infant's blood remained incoagulable for thirty-six hours.

The entire procedure took only ninety minutes and was carried out with facility. This contrasts sharply with our previous experience[10] without the aid of heparin, when only with considerable difficulty, and by tapping numerous vessels including the radial artery, did we succeed in withdrawing the required amount of blood after three hours.

SUMMARY

A simple technique, involving the use of heparin, is described for performing complete exchange transfusions in newborn infants, a procedure which may be indicated in severe cases of icterus gravis.

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PERMEABILITY OF THE HUMAN PLACENTA TO ISOANTIBODIES

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IT HAS been shown that Rh-negative individuals sensitized to the Rh factor may produce either or both of two sorts of antibodies, namely, Rh agglutinins or Rh-blocking antibodies.[1, 2] The Rh agglutinins can be demonstrated by direct tests in saline media, while the Rh-blocking antibodies can be demonstrated by either the so-called blocking test performed in a saline media or directly by the conglutination reaction.[3, 4] The Rh agglutinins behave as if they are bivalent (or multivalent) in the chemical sense, and in tests performed in saline media agglutination occurs directly, presumably because the antibody molecules link the red cells together.[5, 6] On the other hand, the blocking antibodies behave as if they are univalent (therefore the alternative name glutinins for these antibodies) and coat the red cells, preventing the action of the Rh agglutinins in the so-called blocking test.[1] The Rh-blocking antibodies or glutinins can be demonstrated directly in tests performed in the presence of concentrated plasma or serum, because these media seem to contain a third component, conglutinin, probably a colloidal aggregate of plasma proteins analogous to the third component in hemolytic systems.[3]

This hypothesis leads to the concept that Rh agglutinins are larger molecules than Rh glutinins (blockers), which, if true, has important implications in the pathogenesis of erythroblastosis fetalis.[7] One would expect that the univalent Rh antibodies (blockers) should be capable of traversing the placenta more readily than bivalent Rh antibodies (agglutinins). This hypothesis is difficult to test in cases of erythroblastosis due to Rh sensitization, because any Rh antibody passing into the fetal circulation would be immediately adsorbed by the infant's Rh-positive cells and, therefore, would not be demonstrable in the infant's serum except where excessive amounts (more than enough to coat all the erythrocytes) have been acquired from the mother. Nevertheless, we have succeeded[3, 8] in demonstrating the presence of univalent Rh-antibody blockers (or glutinin) coating fetal and infant's red cells and also present in excess in infant's serum for periods up to a month after birth,[3] indicating that these antibodies do gain access to the fetal circulation more readily than Rh agglutinins.

The idea suggested itself to test the hypothesis by comparing the alpha and beta antibody titers in maternal and newborn infant's sera. For this purpose, normal women with normal infants were not suitable, because the maternal serum in such instances would be expected to contain only natural alpha and/or beta antibodies.[9] These sera would therefore be expected to contain

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only agglutinins (bivalent antibodies), not glutinins (univalent antibodies), because the latter occur only in immune sera. Material suitable for testing the hypothesis could only be obtained from cases of erythroblastosis fetalis caused by A-B sensitization.[10] The purpose of this report is to present the results of alpha and beta antibody studies on the mothers and infants of two such cases.

CASE REPORTS

CASE 1.—The clinical details of this case have been described in full in another paper.[10] The infant, a first-born male infant, developed deep jaundice and mild anemia immediately after birth. The jaundice disappeared within ten days, and the infant recovered spontaneously and completely. This is the typical story of mild erythroblastosis due to A-B sensitization (icterus precox).[10, 11] The father of the patient belonged to group A_1NRh_1rh, the mother to group $OMNRh_1Rh_1$, and the patient to group $A_1MNRh_1Rh_1$.

CASE 2.—The patient, a male infant, was the third child. The first child, born seven years previously, developed severe erythroblastosis within twenty-four hours after birth. It was treated first by intramuscular injections of maternal serum (the worst possible treatment according to present knowledge but a treatment which had been recommended for this condition in 1939). Thereafter the infant was given repeated transfusions of 75 to 100 c.c. of whole citrated blood (fourteen transfusions in all) but finally died at the age of four months, still deeply jaundiced. The second infant, born in 1941, was apparently normal at birth and was given 50 c.c. of maternal serum intramuscularly, presumably for purposes of prophylaxis. Shortly after this the infant developed jaundice and anemia. At this time the role of the Rh factor in erythroblastosis had just been discovered, and the child was transfused empirically with Group O, Rh-negative blood. Complete recovery followed three such transfusions of a total 200 c.c. of blood. The grouping tests on the parents and the living child were as follows: father, $A_1MNRh_1Rh_2$; mother, $OMNRh_1Rh_1$; child, $A_1MRh_1Rh_1$. These findings and titrations of the alpha and beta antibodies in the maternal serum indicated that sensitization of the mother to the A factor, and not to the Rh factor, was responsible for the occurrence of erythroblastosis in the first two children of this family. The second infant recovered not because the blood donor used was Rh negative but because he belonged to Group O. On the other hand, the first infant died probably because of the persistent use of homologous Group A blood. It was anticipated before the birth of the third child, our patient, that this infant also would be erythroblastotic. Therefore, immediately after delivery, the cord was clamped and 10 c.c. of Witebsky's solution of A and B group substances were injected into the infant through the vessels of the umbilical cord. As expected, the infant proved to be jaundiced, and the blood smear showed as many as ten normoblasts and erythroblasts per oil immersion field. A mild anemia developed on the second day, and the infant, who weighed only 5 pounds, was given a transfusion of the washed red cells from 50 c.c. of the mother's blood together with 10 c.c. of the A and B group substances. The infant recovered without further treatment and is now 3 months old and thriving.

Comparative titrations of the alpha and beta antibodies in the sera from Cases 1 and 2 were carried out by the agglutination and conglutination techniques,[12] and the results are shown in Table I.

In the titrations carried out by the agglutination technique, it was found that the ratio of the antibody titers of the maternal and infants' sera was 8:1 in Case 1 and 64:1 in Case 2. These ratios compare favorably with those previously reported by Wiener and Silverman[13] for alpha and beta agglutinins in normal pregnancy. On the other hand, in tests carried out by the conglutination technique, the ratios of the titers approximated unity. The reason

TABLE I. COMPARISON OF THE ALPHA AND BETA ANTIBOD

CASE NO.	TECHNIQUE OF TESTS	TEST CELLS	DILUTIONS OF MATERNAL SERUM									TITERS
			UNDILUTED	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
1*	Agglutination	A_2	++±	++	++	+±	+	−	−			16
		B	+++	+++	+++	++±	++	+	−	−		32
	Congluti-nation	A_2	++±	++±	++	+±	+	−	−			16
		B	+++	+++	++	++	++	++	+±	+	−	128
2†	Agglutination	A_2	Hem.	++	++±	++±	++	+±	+	−		64
		B	Hem.	++±	+++	++±	++	+±	+	−		64
	Congluti-nation	A_2	++±	+++	++±	++	++	++	+±	±	−	96
		B	Hem.	++±	++±	++	++	++	+±	+	−	128

Hem., Complete or almost complete hemolysis. Plus signs indicate degree of clumping (agglutination or conglutination). The tests were set up in small tubes in the usual manner; readings were taken after one hour's incubation in the water bath at body temperature.

*Mother, Group O; child, Group A. The maternal and the infant's blood samples in this case were both collected forty-eight hours after delivery.

†Mother, Group O; child, Group A. The maternal blood sample in this case was collected during labor; the infant's blood sample from the umbilical cord immediately after delivery.

for the discrepancy is that the two tests measure two different sorts of antibodies. The former test detects bivalent antibodies (agglutinins), while the latter test also detects univalent antibodies (glutinins). These results demonstrate, therefore, that glutinins traverse the placenta far more readily than agglutinins, in accordance with the expectations under the hypothesis that the former are comprised of smaller molecules.

It is of interest to point out that the serum of the infant of Case 2 caused hemolysis in the agglutination test despite its low titer in saline media. This may serve to explain the observation that the isohemolysin titer is not perfectly correlated with the isoagglutinin titer, because these two reactions are apparently due to two different sorts of antibodies. The tendency of isohemolysis to occur in high-titered agglutinating serum may be explained by the fact that such sera would be more likely to contain immune antibodies.

The fact that in both cases described in this paper first-born infants were erythroblastotic calls for some comment. As has been pointed out elsewhere,[7] fetal red cells and fetal products probably gain access to the maternal circulation principally during labor and only rarely during pregnancy. Thus, maternal sensitization by pregnancy would ordinarily occur only at parturition and too late to affect the first infant. Therefore, for the first-born to be affected, some mechanism of isosensitization other than by the pregnancy itself would have to be postulated. In the first case, the patient stated that two years previously she had had an ovarian tumor removed and was given several post-operative transfusions of commercial, pooled human plasma. Since it has been found that such transfusions stimulate a rise in the alpha and beta isoagglutinin titers, this is a satisfactory explanation for the existence of sensitization in the first case. With regard to the second case, the source of the sensitization could not be traced as definitely. However, it should be pointed out that A-like and B-like antigens are ubiquitous in nature, so that there would be many opportunities for A-B sensitization to occur in the course of a lifetime.[14] For example, such antigens are present in horse serum and pig

TITERS IN MATERNAL SERUM AND NEWBORN INFANT'S SERUM

	DILUTIONS OF NEWBORN INFANT'S SERUM										RATIO OF TITERS
TITER	UNDILUTED	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	TITER	
16	–	–	–							0	–
32	+++	++	+	–	–					4	8
16	–	–	–							0	–
128	+++	+++	++	++	++	++	+±	+	–	128	1
64	–	–	–	–						0	–
64	Hem.	–	–							1	64
96	–	–	–	–						0	–
128	Hem.	++±	++±	++	++	++	+±	±	–	96	1.3

pepsin, so that injections of therapeutic horse serum or diphtheria toxin-antitoxin could bring about A-B sensitization. Moreover, such antigens are present in many bacteria and animal parasites,[15] so that certain infections could cause A-B sensitization. The second patient did give a history of a severe attack of pneumonia several years before her first pregnancy, and possibly this was the source of her sensitization.

It may seem somewhat puzzling that in both cases the univalent beta antibodies were more readily demonstrable in the maternal serum by the conglutination method than were the univalent alpha antibodies. The following plausible explanation for this apparent paradox suggests itself. It would be expected that univalent antibodies in general would continue to pass through the placenta from mother to fetus until their concentration in the fetal circulation became equal to that in the maternal circulation, thus setting up an equilibrium. In the case of the beta antibodies, such an equilibrium could be reached quickly; but in the case of the alpha antibodies, the antibodies would be neutralized or absorbed by group substances in the Group A fetus' body, delaying the attainment of equilibrium and at the same time gradually depleting the mother's body of the alpha antibodies.

SUMMARY

Two cases of erythroblastosis due to A and B sensitization are described. Comparative titrations by the agglutination and conglutination techniques of the alpha and beta antibodies in the maternal and infants' sera indicate that glutinins (univalent antibodies) traverse the placenta more readily than agglutinins (bivalent antibodies). This supports the hypothesis that glutinins (or blockers) are comprised of smaller molecules than agglutinins.

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THE QUANTITATIVE DETERMINATION OF A PEPSIN-LIKE SUBSTANCE IN THE URINE OF NORMAL INDIVIDUALS AND OF PATIENTS WITH PERNICIOUS ANEMIA

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THE first report of a proteolytic substance in urine was made in 1861 by Brücke,[1] who noted that fibrin was digested when incubated with urine. Similar experiments were performed by Grützer[2] in 1887, Gehrig[3] in 1886, and Hoffmann[4] in 1887. The character and origin of this ferment were studied further by Leo[5] in 1885, Schnapauf in 1888,[6] and Loeper and Esmonet[7] in 1908. On the basis of this work, there developed two theories: first, that the enzyme was pepsin and was derived from the intestine; second, that the active principle was a proferment probably secreted directly into the blood stream by the gastric mucosa. Frouin,[8] in 1904, working with a gastrectomized dog and another in which the stomach had been isolated with the blood supply intact, concluded that urinary pepsin is of gastric origin, that it enters the blood stream at the level of the stomach, and that no resorption occurs in the intestine. After this simple and critical experiment, the progress and validity of investigation in this field were hampered by analytic methods not sufficiently accurate to insure other than qualitative results, and attempts to relate the urinary ferment quantitatively with gastric secretion of pepsin, either in health or in disease, proved confusing.

It was not until 1924 that Gottlieb[9] devised an accurate method for the simultaneous study of pepsin in the stomach, in the blood, and in the urine. His method is not fully described, but it consisted essentially of digestion of edestin with comparison to the activity of a known solution of Armour's pepsin. Applying this method to a small group, Gottlieb reported that high gastric acidity was associated with a significantly higher urinary pepsin than was low gastric acidity. He also noted a correlation between gastric and urinary pepsin. Gottlieb further reported that alkalinization of the urine to a pH of 8 or 9 did not inactivate the enzyme and concluded that the substance was pepsinogen. Three years later Teschendorf[10] studied the pepsin content of the gastric juice and urine in patients with achylia. Here again, the number of cases was small and the results conflicting.

The purpose of the present paper was to study further the excretion of urinary pepsin in normal individuals and in patients with achylia (pernicious anemia) with the use of improved methods. We found that the modification of the Anson and Mirsky method (Beazell and associates[11]) could be satisfactorily adapted for the determination of urinary pepsin. This method was employed by Bucher[12] in a preliminary report of a study on young women. Our results show

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a striking decrease in the pepsin or pepsin-like substance in the urine of patients with pernicious anemia as compared to normal individuals.

METHODS AND MATERIAL

All determinations were made on fasting morning urine specimens. The method of determining pepsin activity involves the incubation at 37° C. of 5 c.c. of a known reproducible hemoglobin substrate, buffered at a pH of 1.7, with 1 c.c. of urine for sixteen hours. To prevent bacterial growth, the mixture is overlayered with toluene. After incubation the solution is treated with 10 per cent trichloracetic acid to precipitate the undigested hemoglobin. The phenolic digestion products in the filtrate, presumably chiefly tyrosine, are than quantitatively determined by the photoelectric colorimeter and compared with a known solution of tyrosine. Because phenolic compounds are normally found in the urine, a blank consisting of 1 c.c. of urine, similarly treated but previously inactivated by heating for five minutes in a boiling water bath, is concomitantly assayed, and the results are subtracted from the reading of the active sample. The peptic activity is expressed as milligrams of tyrosine produced by 1 c.c. of urine. The tyrosine equivalent has been carefully investigated throughout the range of concentrations with which we are dealing and has been found to bear nearly a straight line relationship.

The normal series consisted of eighty-two individuals comprising both sexes and all age groups from the second to the eighth decades. The younger subjects were for the most part medical students and clinic personnel, while the older group were ambulatory and hospital patients who were in comparatively good health and had no symptoms referable to the gastrointestinal tract. Gastric analyses were not performed in these persons.

The pernicious anemia series consisted of forty-one patients of both sexes from the hematology clinic. All of these patients were receiving parenteral liver therapy and were in complete remission. Originally they had had the typical blood picture of pernicious anemia, had shown complete achlorhydria following histamine stimulation, and had responded specifically to liver therapy.

RESULTS

Individual values for the peptic activity of urine are plotted against the age of the subjects in the spot curve (Fig. 1). The normal series have a wide range of values which is compatible with the expected variation in gastric function seen in an unselected group. The values ranged from 0.7 to 4.4 mg. with an average of 2.4 mg. tyrosine per 1 c.c. urine for sixteen hours. Among the patients with pernicious anemia, the values ranged from 0 to 1.0 mg. with an average of 0.5 mg. Taking 1.0 mg. as the maximum value in pernicious anemia, only five of the eighty-two normal subjects had a peptic activity at or below this level. Gastric analysis might possibly have shown diminished gastric function in these five patients.

A decrease in urinary pepsin might have been anticipated in the advanced age group; a clear-cut correlation, however, could not be made (Fig. 1), and plotting by decades gave results similar to those obtained by Helmer, Fouts, and

Zerfas[13] in studies of gastric enzymes. A larger series must be studied before such a relationship can be excluded.

The effect of specific gravity on the urinary peptic activity was negligible. Twelve normal urine specimens with the highest tyrosine values had an average specific gravity of 1.019 as compared to 1.016 in the twelve normal urines with the lowest tyrosine values. The acidity of the urine was likewise unrelated to the peptic activity. Many specimens with a pH of 7.0 or over had normal tyrosine values. This is in agreement with the idea that the enzyme in urine is a proferment or pepsinogen.

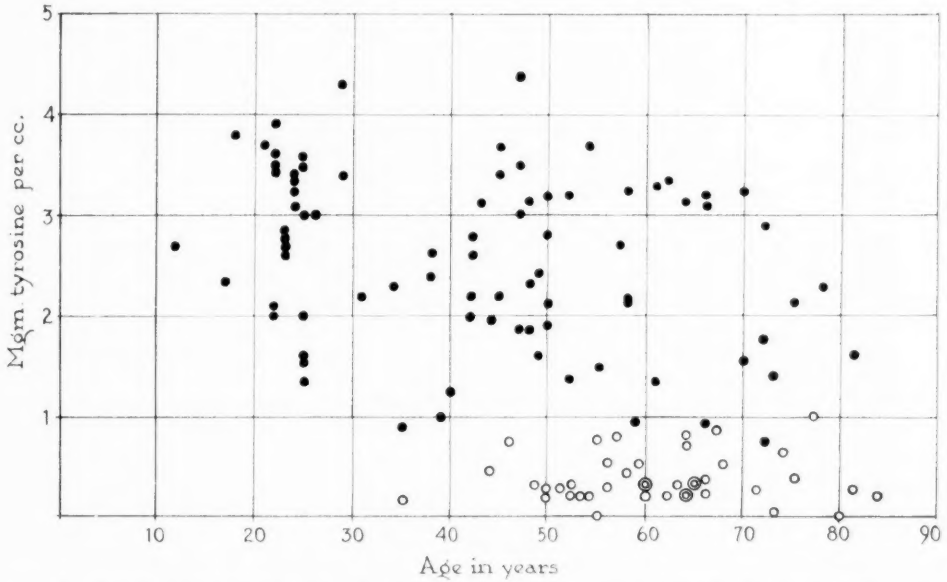

Fig. 1.—Relation of tyrosine value to age. Dots indicate normal individuals; open circles, patients with pernicious anemia.

COMMENT

The term pepsin-like substance has been used in the title because of the possibility that the enzymatic action of the urine was not due to pepsin. However, the facts that (1) the digestion of hemoglobin solution occurs at a low pH, (2) the enzyme concentration of urine of patients with achylia gastrica is low as compared to normal individuals, and (3) the substance is not irreversibly inactivated in a neutral solution strongly suggest that the enzyme is related to pepsin and occurs normally as pepsinogen. Further evidence for this conception is presented in the older literature.[8, 9]

Although determinations of the gastric pepsin were not made in our subjects, it might be inferred that the quantitative urinary pepsinogen roughly parallels the peptic activity of the gastric juice. Patients with pernicious anemia, who are known to have a marked diminution of gastric pepsin,[13] had a correspondingly low urinary pepsin. Patients with achlorhydria following histamine stimulation but not having pernicious anemia are known to have some decrease in the gastric pepsin but not to the extent observed in pernicious

anemia.[14] We studied the urinary pepsin in fourteen women patients with true achlorhydria, all of whom had recovered from an iron deficiency anemia. The peptic activity expressed as tyrosine ranged from 0.3 to 3.2 mg. with an average of 1.2 mg. (average for normal subjects, 2.4 mg.). Eight of the fourteen patients had values above 1 mg., the upper limit seen in pernicious anemia. Further evidence thus is offered for a quantitative relation between gastric and urinary pepsin.

The extremely low values for peptic activity of the urine that were observed in patients with pernicious anemia might make the test applicable for clinical purposes. The method is simple and is performed on a single fasting urine specimen. In the forty-one patients with pernicious anemia, the peptic activity of 1 c.c. urine expressed as milligrams of tyrosine showed values of 1 mg. or less, whereas only 6 per cent of eighty-two normal persons* fell within this range. Therefore, in a patient suspected of having pernicious anemia, a tyrosine level above 1 mg. would be good evidence against this diagnosis.

SUMMARY

1. Quantitative estimations of a pepsin-like substance in the urine were made by a modification of the Anson and Mirsky method.

2. The peptic activity of the urine was markedly diminished in patients with pernicious anemia as compared to normal subjects.

3. The possible diagnostic application of the test is discussed.

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*No gastric analyses were performed on these individuals.

HEMOCHROMATOSIS

Review of Literature and Presentation of a Case Without Pigmentation or Diabetes

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HEMOCHROMATOSIS is a rare disorder of iron metabolism occurring predominantly in middle-aged men, characterized by a clinical triad of hepatic enlargement, diabetes, and pigmentation of the skin. This case, occurring in a 14-year-old white boy without pigmentation of the skin and diabetes, is being presented because of its atypical clinical features.

CASE REPORT

A 14-year-old white boy was admitted for the first time to the University Hospital on Feb. 1, 1944, with the chief complaint of upper respiratory infection. For the past six years he had been anemic and had been treated with liver and iron. The anemia was of the hypochromic type and gradually progressed. For six months before the first admission he had complained of progressive weakness and fatigue. A few days before this admission he developed an upper respiratory infection with postnasal drip, hacking cough, and frontal headache. On physical examination the spleen was palpated three fingerbreadths below the right costal margin and was slightly tender. The blood count showed: red blood cells, 2.5 million per cubic millimeter; hemoglobin, 3.9 Gm. per 100 c.c.; reticulocytes, 1.2 per cent; and white blood cells, 4,000 per cubic millimeter with 64 per cent neutrophiles, 27 per cent lymphocytes and 9 per cent monocytes. There were 350,000 platelets per cubic millimeter. The fragility of the red blood cells was normal. A sternal puncture revealed hyperplasia of the erythroid elements. He was Rh negative. The results of an adrenalin test were interpreted as showing excessive sequestration of red blood cells, white blood cells, and platelets. On the eighteenth hospital day a splenectomy for Banti's disease was performed. At the time of the operation the liver was enlarged but did not appear to be cirrhotic. Microscopic sections of the spleen showed reticulum-cell hyperplasia with fibrosis and dilatation of the sinusoids; hemosiderin was present in increased amounts. Postoperatively the red blood cells were stabilized at 4.5 million per cubic millimeter and the white blood cells at 7,000 per cubic millimeter. After an uneventful course the patient was discharged on the fourteenth postoperative day.

The second admission occurred three months after the first. At this time the patient complained of weakness with anorexia and nausea. He was pale, weak, and listless. The liver was not palpable. The admission blood count showed: red blood cells, 1.75 million per cubic millimeter; hemoglobin, 3.8 Gm. per 100 c.c.; reticulocytes, 1.6 per cent; and white blood cells, 15,000 per cubic millimeter with 60 per cent neutrophiles and 40 per cent lymphocytes. The patient was given four transfusions. The red blood count rose to a maximum of 2 million per cubic millimeter and the hemoglobin to 8 Gm. per 100 c.c. He was discharged on the seventh hospital day.

The third admission was two months later. The patient was pale, poorly nourished, and somewhat icteric. The liver was palpable two fingers below the right costal margin. On three successive days he received 1,000 c.c. of suspended red blood cells. No blood counts were recorded for this admission. The patient was discharged greatly improved.

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The fourth and final admission occurred two and one-half months later, at which time the patient complained chiefly of abdominal pain. A week before admission he was noted to have a mild fever. Three days later right upper quadrant discomfort appeared and later became a definite pain radiating into the back and shoulders. The pain grew steadily worse. The patient became nauseated and vomited. Physical examination revealed a pale, poorly nourished, young white boy complaining of abdominal distress, sitting up in bed, and leaning over a pillow pressed against his abdomen. The temperature was 102° F.; pulse, 120; respirations, 30; and blood pressure, not recorded. The eyes, ears, nose, and throat were normal. The lungs were clear. The heart was enlarged to the left anterior axillary line and two fingerbreadths to the right of the sternum. A blowing systolic murmur was heard at the apex, transmitted over the precordium. An aortic systolic murmur was also present. The abdomen presented a well-healed splenectomy scar. The liver was enlarged three fingerbreadths below the right costal margin and was markedly tender. The extremities were normal and the reflexes physiologic.

The admission urine was negative. The admission blood count showed: red blood cells, 2.5 million per cubic millimeters; hemoglobin, 6 Gm. per 100 c.c.; reticulocytes, 3 per cent; and white blood cells, 30,000 with 70 per cent neutrophiles, 22 per cent lymphocytes, and 8 per cent monocytes. The platelets numbered 2 million per cubic millimeter. The succeeding blood counts showed similar results, except that the platelets steadily declined to 600,000. The blood urea nitrogen was normal. The icterus index was 3. Total proteins were 4.9 Gm. per 100 c.c. Blood cultures were negative, as were agglutinations for typhoid, paratyphoid, and brucellosis. A flat plate of the abdomen showed no pathologic changes.

The temperature varied from 99.4° F. to a terminal value of 105°. The abdominal pain radiated to the back and shoulders, persisting until death, and distention appeared. Large amounts of flatus and formed stools were obtained by enema. Several whole blood transfusions were given. Vomiting of undigested food occurred. Later the emesis became thick, mucoid, and yellow. Wangensteen suction was instituted, and dark red drainage was obtained. The distention, however, was not relieved. A paracentesis yielded 1,400 c.c. of dark orange fluid. Bleeding from the rectum occurred. The patient became cyanotic with a weak and thready pulse. He died on the ninth hospital day. The clinical diagnosis varied from subacute bacterial endocarditis to acute surgical abdomen secondary to diseased gall bladder or appendix.

Autopsy.—The body was that of a well-developed, fairly well-nourished 14-year-old white boy weighing approximately 120 pounds. The skin and mucous membranes were pale. A well-healed left paramedian incision was noted over the abdomen. The abdominal cavity contained about 2 liters of a thin bloody fluid. Numerous firm fibrous adhesions were present beneath the previous operative site and between the liver and diaphragm. There was moderate distention of the loops of the small bowel. Most of the coils of small intestine in the left half of the abdomen were bluish, discolored, and gangrenous in appearance. Those in the right half of the abdomen were not so deeply discolored and were covered with a recent fibrinous exudate. The heart weighed 250 Gm. and was grossly normal. The right lung weighed 330 Gm. The lower one-third of the lower lobe was atelectatic. The mid-portion of the lower lobe presented a fibrocaseous Ghon tubercle about 1.5 cm. in diameter. The left lung weighed 210 Gm. and was grossly normal. The spleen was surgically absent. Five small splenules, each about 8 mm. in diameter, were found in the greater omentum just beneath the stomach on the left side. The liver weighed 2,350 Gm. There were marked fibrous adhesions covering the capsule of the anterior part of the left lobe. The cut surfaces had a yellow-orange color and were broken up into multiple small nodules, each about 2 mm. in diameter and surrounded by narrow pink areas. The portal vein and its branches were filled with a soft, whitish-gray, firmly adherent, thrombotic material. Further dissection revealed complete thrombosis of the portal vein and all the large branches of the superior mesenteric vein. The splenic vein and artery were atrophic. The gall bladder was essentially normal. The pancreas weighed 100 Gm. and was somewhat brown in color. The esophagus was grossly normal. There were no varices. The stomach was grossly normal. There was

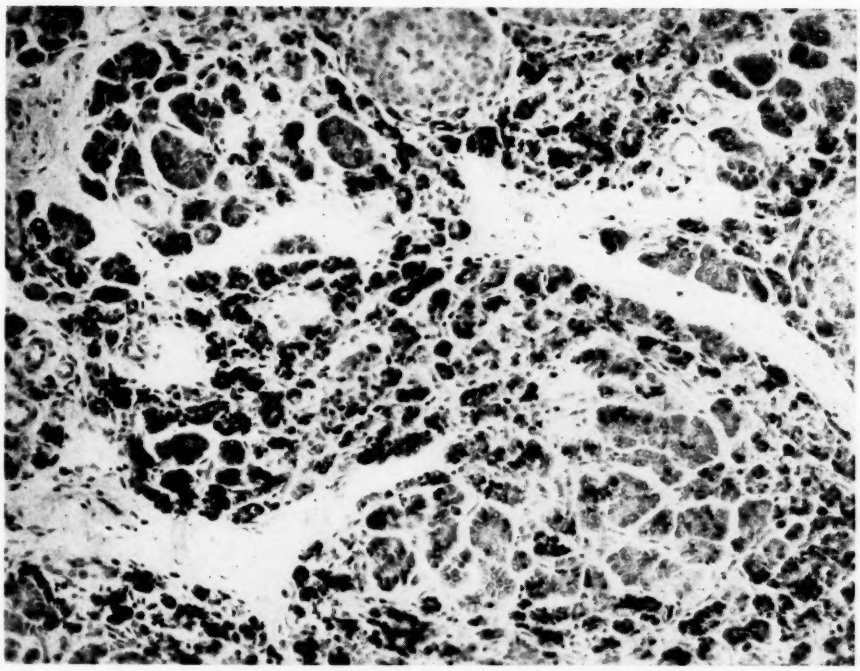

Fig. 1.—Pancreas. Section shows diffuse hemosidero-fibrosis with practically no involvement of islets. (Iron stain.)

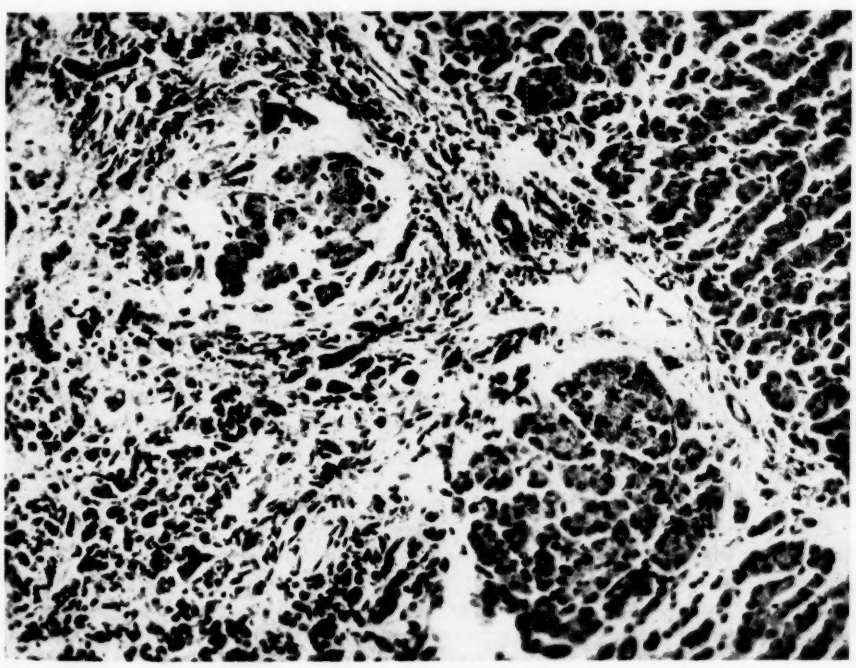

Fig. 2.—Liver. Section shows cirrhosis and hemosiderosis. The pigment is deposited in greater quantities in large mononuclears in the portal canals. It is also present in large amounts in the parenchymal cells and Kupffer cells. (Iron stain.)

a hemorrhagic infarction of the lower two-thirds of the small bowel with lesser involvement of the upper portion. The loops were heavy and filled with a bloody material. The wall was hemorrhagic. The mesenteric fat was edematous and thick, measuring up to 3 to 4 cm. in thickness. Examination of the main superior mesenteric artery and its branches failed to reveal any evidence of thrombus. The fairly soft and yellow-gray thrombus in the superior mesenteric vein was previously described. The adrenals showed lipoid depletion. Each kidney weighed about 190 Gm. They were pale and soft. The bladder, ureters, and sex organs were normal. The aorta showed no evidence of atherosclerosis or syphilis. The bone marrow was red. The head and spinal cord were not examined.

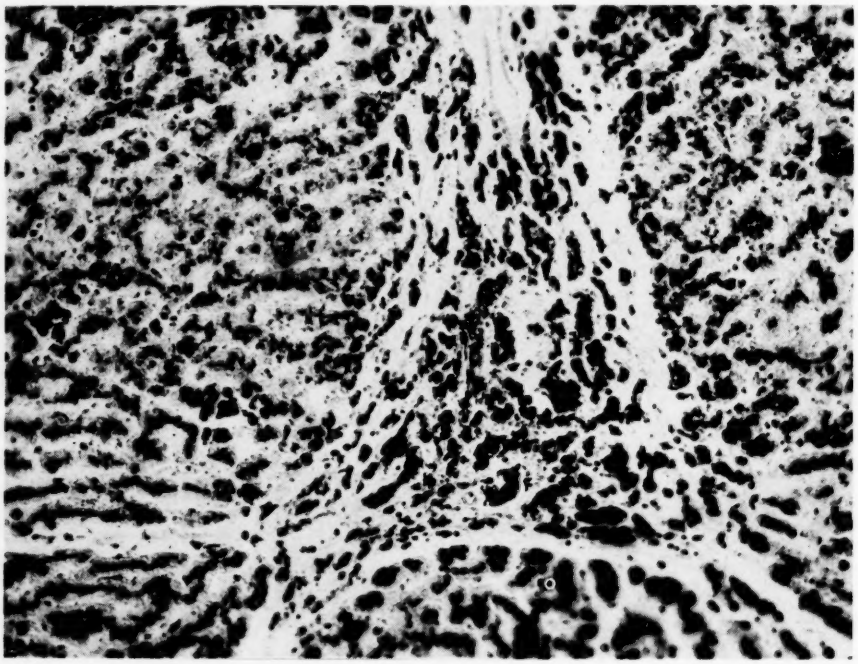

Fig. 3.—Liver. Section is similar to that illustrated in Fig. 2 and shows cirrhosis and pigmentation. Large quantities of iron pigment are readily seen with routine stains such as this. (Hematoxylin and eosin.)

Microscopic Findings.—The heart showed parenchymatous degeneration. The lungs showed thickening and congestion of the alveolar septa and a few small arterial thrombi. The splenule showed congestion, reticulum-cell hyperplasia, and hemosiderosis. No lymphoid tissue was noted in the splenules. The liver showed a portal type of cirrhosis with excessive iron pigmentation in the periportal areas, Kupffer's cells, and parenchyma. The greatest amount of iron was in the portal canals where the iron was found mainly in phagocytic macrophages. There was a recent organizing thrombus of the portal vein with periportal chronic inflammation. The pancreas showed patchy degeneration with hemosidero-fibrosis. The islets were uninvolved and were negative for pigment with iron stains. Other changes were fatty infiltration and endarteritis of the splenic artery. The gastrointestinal tract sections showed congestion, edema, and early infarction of the small bowel. The kidneys showed parenchymatous degeneration. Sections of the sex organs revealed no significant histopathologic changes. The bone marrow showed an increase in megakaryocytes. Special stains for iron were made of all organs. Small amounts of iron pigment were also found in epithelial cells of prostate, heart, and in the epithelial cells of the collecting tubules of the cortex. Moderate amounts of iron were found in the outer layers of the cortex of the adrenal. Fairly large

amounts of iron were found in the splenule. Quantitative analysis of the liver showed 2 per cent by weight, or 47 Gm. of iron in the liver. No sections of the skin were submitted for histologic examination.

Final Diagnosis.—Hemochromatosis with hemosidero-fibrosis of the liver and pancreas and generalized hemosiderous; portal cirrhosis of liver; recent thrombosis of portal and superior mesenteric veins with hemorrhagic infarction of the lower three-fourths of the small intestine; atelectasis of the right lower lobe; Ghon tubercle in the lower lobe of right lung; absence of spleen (surgical removal).

DISCUSSION

Diagnosis.—From the point of view of differential diagnosis this case represents several interesting problems. The age of the patient is much younger than the usual recorded age of 45 to 50 years. In the series of Sheldon[1] the earliest recorded age for the appearance of the fully developed disease is 20, although symptoms of the disease did appear at 7, 16, 17, 18, and 19 years of age. Sheldon further states that the occasional early emergency of symptoms may be expected.

The pathologic interpretation of the case is that of hemochromatosis. The diagnosis is based upon the findings of pigment cirrhosis of the liver, fibrosis and pigmentation of the pancreas, and siderosis of other organs.

Two of the three classic signs and symptoms, diabetes and pigmentation of the skin, were absent. In Sheldon's series pigmentation of the skin constituted the first symptom of disease in 25.7 per cent of the cases and was found in 83.8 per cent of his series. It was the first symptom noticed in 40 per cent of the series published by Butt and Wilder.[2] Lisa and Hart,[3] on the other hand, emphasized that a certain number of cases may not show any skin pigmentation and that as many as 75 per cent of early cases will have no evidence of bronzing. Diabetes was found in 25.7 per cent of the patients in Sheldon's series as the initial symptom and was finally present in 70 per cent of his cases. Eighty-six per cent of the Mayo series[2] gave laboratory evidence of diabetes. According to Karsner[4] one out of every 2,000 to 3,000 persons with diabetes show hemochromatosis. Enlargement of the spleen, which in our case was one of the first noted organic changes, is known to occur in hemochromatosis. In Sheldon's series the spleen was enlarged in 60 per cent of the cases, while an enlargement of the liver was diagnosed in 92 per cent of the cases.

A particularly interesting finding in our case was the presence of anemia which preceded the onset of all other symptoms by approximately six years. From the laboratory data we can conclude that it was a hypochromic microcytic anemia with no evidence of marrow hypoplasia. The values for the red blood cells and hemoglobin alone were depressed. The reticulocytes were either normal or moderately elevated. The platelets were normal before splenectomy and greatly elevated after splenectomy. The white blood cells were decreased in number before splenectomy and fluctuated considerably afterward. These findings would support a diagnosis of an iron deficiency anemia as against an aplastic type of anemia. Only six cases of Sheldon's series showed a red blood count below 2.5 million per cubic millimeter. Anemia associated with hemochromatosis has been observed by other authors, and the combination of both

conditions has been considered more than accidental by Zeltmacher and Bevans.[5] However, their case, as well as those of Kark,[6] Sturgis,[7] and Mackey,[8] and the three cases of Bomford and Rhoads[9] were aplastic in type.

The cause of death in our case must be considered quite unusual and may be one of the late complications of splenectomy in portal obstruction. The thrombosis was not accompanied by a pyogenic process in the portal system. It is interesting that we had the opportunity at operation to study the spleen at a stage where no evidence of pigment cirrhosis was noted. Microscopically the spleen showed an increased amount of iron pigment together with changes typically associated with the spleen in liver cirrhosis.

Pathogenesis.—Sheldon, who made an exhaustive study of the disease, believes that hemochromatosis is a congenital disease and consists chiefly of a fundamental disorder in the iron metabolism with accumulations of small amounts of pigment over a long period of time. Consequently, it takes 40 to 50 years for the emergency of symptoms, although occasionally symptoms occur at an earlier date. In his series of 311 cases there was no evidence of increased hemolysis, such as increased icterus index, reticulocytosis, or increased bilirubin excretion in the urine, nor was there any evidence of the action of a toxin. The majority of writers on this subject concur with this opinion. Rosenthal[10] believes that the fundamental disturbance is the inability of the liver to change ferric to ferrous iron. Mallory's[11] work on experimental animals led him to believe that intoxication with copper with resultant destruction of erythrocytes and liberation of iron from hemoglobin is an important factor in the production of hemochromatosis. Smith and Gault[12] suggest that hemochromatosis might be analogous to von Gierke's disease, the iron entering into the cell normally and being transformed into a form incapable of excretion.

An interesting theory has been suggested by a group of authors[5] who described hemochromatosis in association with aplastic anemia. They believe that in those cases observed after they have been kept alive by a large number of blood transfusions increased hemolysis may be the etiologic factor of hemochromatosis, while the cause of anemia is probably obscure. It is suggested that the iron derived from the destruction of intrinsic and transfused blood is deposited in a cirrhotic liver, thus producing a picture indistinguishable from hemochromatosis. As the pancreas becomes involved in this process, diabetes might develop.

Whether hemochromatosis can be produced by an increased amount of blood transfusions in various anemic conditions has not been definitely answered. Sturgis expresses the opinion that in his case hemochromatosis did develop after 137 blood transfusions. Mackey and Kark likewise believe that the hemochromatosis in their cases is directly related to the amount of iron administered in transfusions. Of the other cases of aplastic anemia developing hemochromatosis, Bomford and Rhoads make no direct statement as to the relationship of hemochromatosis to multiple transfusions, while Zeltmacher and Bevans are unable to account for the total amount of iron found at autopsy on the basis

of transfusions alone. Kilduffe and DeBakey[13] state that occasionally patients who receive transfusions over prolonged periods of time develop hemochromatosis, but they do not give details. However, others experienced in the consequences of blood transfusion would not generally accept such etiologic relationship.[14-17] Experimental attempts to reproduce hemochromatosis by repeated transfusions, by hemolytic agents, and by injection hemoglobin or dialized iron have not been successful.[18-20]

In our case the patient received a total of 6 liters of blood over a nine months' period, or 2.75 Gm. of iron. The amount of iron recovered from the liver was 47 Gm. From these calculations it is clear that the pigmentation cannot be explained by the retention of transfused iron.

A recent article by Gillman and Gillman[21] from South Africa, on the structure of the liver in pellagra, is most interesting and if confirmed would constitute a new approach to the study of hemochromatosis. On the basis of 400 liver biopsies in 120 patients with pellagra, they conclude that hemochromatosis is one of the commoner sequelae of pellagra (15 per cent of the cases). The hemosiderin and hemofuscin pigments are formed in association with changes in the mitochondria, ultimately leading to the disruption of the mitochondria. This profound disturbance of intracellular metabolism is related to some forms of malnutrition. Hemochromatosis is therefore considered as an acquired rather than as a congenital disease. Further observations, based on a series of 700 livers from autopsies in sudden and violent deaths (unreported), lead them to believe that hemochromatosis is a common disease in Africans. They attribute this to the totally inadequate diets of these natives.

There is inadequate information to discuss our own case from a nutritional point of view in line with Gillman and Gillman's recent concept. Notes on the first two admissions described the patient as being well nourished and on the last two admissions as being poorly nourished. The autopsy protocol describes the patient as being fairly well nourished.

SUMMARY AND CONCLUSION

1. An atypical case of hemochromatosis without pigmentation of the skin and diabetes as occurring in a 14-year-old white boy is presented.

2. The diagnosis is based upon the findings of pigment cirrhosis of the liver and pancreas and siderosis in other organs.

3. A particularly interesting feature in our case is the presence of a severe anemia which preceded the onset of other symptoms by approximately six years. From the laboratory data we classify this as a hypochromic microcytic anemia of the iron deficiency type.

4. In the more recent literature the association of hemochromatosis with cases of aplastic anemia has been noted. The possible relationship between hemochromatosis and the multiple transfusions given to these cases has been suggested. At the present time we do not believe that this question has been definitely answered.

5. No such relationship is present in our case. A total of 2.75 Gm. of iron were contained in the transfused blood. The liver at autopsy contained 47 Gm.

6. In a recent paper Gillman and Gillman propose a nutritional basis for the pathogenesis of hemochromatosis.

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LABORATORY METHODS

A SIMPLE METHOD FOR PERFORMING A WASSERMANN TEST ON ANTICOMPLEMENTARY SERUM

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IN ALL clinical laboratories serologists have obtained anticomplementary reactions in the performance of the Wassermann tests. The common practice in such cases is to report the test as anticomplementary and to request that another specimen of blood be taken. Usually, the next specimen is found to be satisfactory. There are also some cases in which anticomplementary reactions are obtained on several successive specimens of serum.

An attempt was made to study these tests and to devise a simple method for performing a Wassermann test on anticomplementary serum, in order to be able to report the test as negative, doubtful or positive and to eliminate the necessity of taking further specimens of blood from the patient.

Since the criterion for the anticomplementary reaction is found in the control tube, where no antigen is added, it becomes apparent that the patient's serum has the ability to "fix" complement by itself. It was found expedient, therefore, to saturate the patient's serum with complement by incubation with undiluted complement at 37° C. and then to destroy the excess complement by inactivating for thirty minutes at 56° C. At this point the serum is ready for the Wassermann test. The ability of the serum to "fix" complement has now been thoroughly satisfied, and further fixation in the control tube (without antigen) is no longer possible. The usual amount of complement is added to all of the tubes in the performance of the Wassermann test on the complement-saturated serum.

This conception has been amply borne out experimentally. The tests were performed on 200 anticomplementary serums over a period of about fifteen months. Included with each setup of anticomplementary bloods were known negative and positive serums as controls for the method employed. Fresh specimens of blood were also obtained from those patients having anticomplementary reactions in order that routine Wassermann and Kahn tests could be performed as additional controls for the complement-saturation method.

METHOD

To 2 c.c. of anticomplementary serum add 0.3 c.c. of undiluted complement and place in 37° C. water bath for thirty minutes. Remove and place in 56° C. water bath for thirty minutes to destroy excess complement. Perform a Wassermann test on the serum in the usual manner. The Kolmer three-tube test was employed in this laboratory.

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It will be found that in the great majority of cases this proportion of serum and undiluted complement will suffice to saturate the serum. However, if an anticomplementary reaction is again obtained, the procedure can be repeated with an additional dose of 0.1 to 0.2 c.c. of undiluted complement.

RESULTS

All bloods that were anticomplementary we were now able to report as negative, doubtful, or positive. In each case the control tube showed complete hemolysis.

The addition of undiluted complement to known negative and positive serum had no deleterious effects. The results on these serums were the same with and without complement saturation.

TABLE I. REPRESENTATIVE SAMPLE OF RESULTS OF COMPLEMENT-SATURATION TESTS ON ANTICOMPLEMENTARY SERUM AND CONTROL WASSERMANN TESTS ON FRESH SERUM AND KAHN TESTS

CASE	WASSERMANN ON ANTICOMPLEMENTARY SERUM	WASSERMANN ON FRESH SPECIMEN OF SERUM	KAHN
1	Positive	Positive	Positive
2	Positive	Positive	Positive
3	Positive	Positive	Positive
4	Positive	Positive	Positive
5	Positive	Positive	Positive
6	Positive	Positive	Positive
7	Negative	Negative	Doubtful
8	Doubtful	Doubtful	Positive
9	Positive	Positive	Positive
10	Positive	Positive	Positive
11	Positive	Positive	Positive
12	Positive	Positive	Positive
13	Positive	Positive	Positive
14	Negative	Negative	Negative
15	Positive	Positive	Positive
16	Positive	Positive	Positive
17	Positive	Positive	Positive
18	Doubtful	Doubtful	Doubtful
19	Positive	Positive	Positive
20	Positive	Positive	Positive
21	Positive	Positive	Positive
22	Positive	Positive	Positive
23	Positive	Positive	Positive
24	Negative	Negative	Negative
25	Positive	Positive	Positive

The Wassermann tests on the anticomplementary serums checked very closely with the Kahn tests, as well as with repeated Wassermann tests performed on fresh serum. In Table I is given a representative sample of the results obtained in this study. It is noteworthy that the majority of serums that were anticomplementary were positive with the method described.

Although the method could be applied to anticomplementary serums that are very badly hemolyzed or greatly contaminated with bacteria, it is not advisable to run these. In such cases it is best to obtain fresh specimens of blood, since this is encountered very infrequently.

SUMMARY AND CONCLUSION

1. A simple procedure has been given which permits the performance of a Wassermann test on anticomplementary serum and which eliminates the necessity of taking further specimens of blood from the patient.

2. The procedure is based on the saturation of the serum with undiluted complement to satisfy the "fixing" properties of the serum. The excess complement is then destroyed, and a Wassermann test is performed in the usual manner.

3. In all cases complete hemolysis was present in the control tube, and the tests were reported as negative, doubtful, and positive.

4. Known negative and positive serums were treated in the same manner as the anticomplementary serums in order to check on the effects of complement saturation. The results of these tests were the same with and without complement saturation.

5. There was excellent correlation between the Wassermann test on anticomplementary serum and the repeated Wassermann test on fresh serum and the Kahn test.

A MECHANICAL DEVICE TO FACILITATE ELECTROCARDIOGRAPHIC READING

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THERE are several mechanical devices in use to facilitate electrocardiographic interpretation. They are based on the idea of applying an indicated scale against the electrocardiogram, merely trying to eliminate time multiplication or counting of millimeters. The need was felt for a new instrument, simple in design, to aid in every possible phase of electrocardiographic reading without the additional use of compasses.

This instrument (Fig. 1) combines the principle of the slide rule and the caliper. It is made of plastic material and is equipped with a sliding tongue, which makes it possible to gauge distances and to compare set distances. It is also equipped with a glass lens of such focal length and size as to collect the light rays from a near-by source of light and thereby to brighten up and magnify the observed area four times, without distorting the millimeter and time lines of the electrocardiogram.

The sliding tongue and the fixed zero indicator have a longitudinal line which can be used as a base line.

The instrument has three scales which read from the right to the left. Scale 1 indicates millimeters; Scale 2 indicates the calculated time intervals; and Scale 3 indicates the calculated heart rates of a regular rhythm.

The reading is taken against the hairline on the cover glass which is mounted in a fixed aluminum frame. The gauging and comparing of distances are obtained directly on the electrocardiogram, between the edge of the wedge-shaped fixed zero-piece (*A*) and the edge of the wedge-shaped end of the sliding tongue (*B*). The scales are impressed upon the top surface of the sliding tongue, directly below the hairline. This eliminates faulty reading due to parallax, both in gauging and reading.

The instrument is applied as follows:

1. *Determination of Regularity.*—The reader is placed on the electrocardiogram with the longitudinal line over the base line. The edge of the fixed zero-piece is set over the peak of the R or S wave, and the edge of the sliding tongue is pulled out to the peak of the next R or S wave. In order to determine the regularity or irregularity of the heart rate, this interval is compared with corresponding intervals in other complexes by moving the set reader to various complexes.

2. *Reading of the Heart Rate.*—The heart rate is obtained directly by taking the reading from the heart rate scale of the R-R or S-S interval against the hairline.

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*Research Assistant in Medicine.

3. *Reading of Time Intervals.*—The reader remains in the same position. The time scale indicates the distances in seconds. The tongue is pulled out to the end of the wave whose time is to be measured, while the fixed zero edge coincides with its beginning. The exact time interval is read against the hairline.

4. *Reading of Millimeter Distances.*—The instrument is placed on the electrocardiogram with the longitudinal line in the direction of the millimeter lines. The reading of the height of the different waves is obtained by moving the sliding tongue from the base line to the apex of the wave and by reading the indicated millimeters against the hairline. Such set intervals can be compared with different complexes simply by moving the reader over to the various complexes.

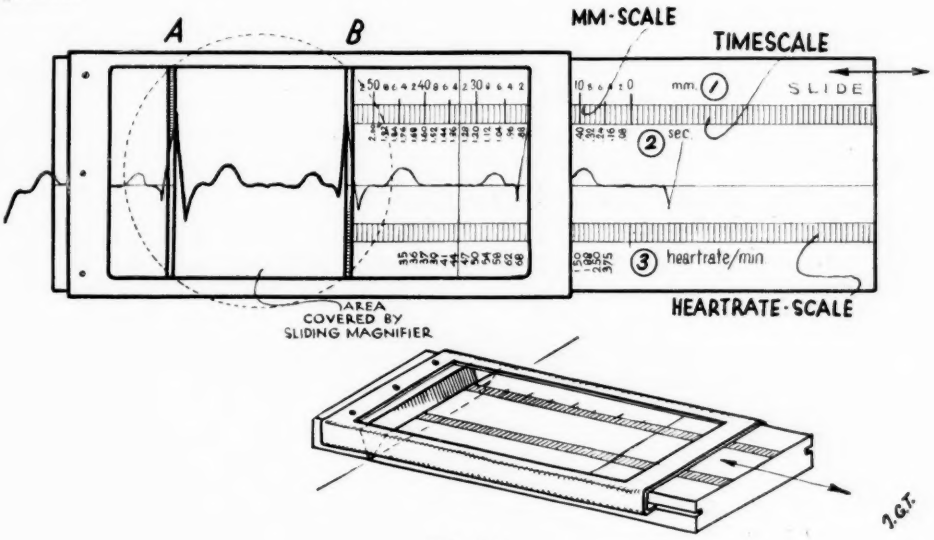

Fig. 1.*

The reader is transparent over its entire area in order to maintain visibility of the full length of the electrocardiogram under scrutiny.

This reader helps to obtain accurate measurements. It aids in compiling numerous data for electrocardiographic research and for diagnosis.

*The instrument will be manufactured by the United Optical Mfg., Corp., New York, N. Y.

AN ELECTROCARDIOGRAPHIC CALIPER CALCULATOR

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Chicago, Ill.

RULES for the direct measurements of heart rate (and cycle length) on the electrocardiogram are based on a fixed reference distance, implying a single and uniform camera speed. This precludes their use for cameras with different speeds and leads to errors in reading when the speed of the camera is not uniform.

The present caliper was developed to take into account such variations in camera speed and to make the instrument adaptable for a wide range of speeds. The caliper is constructed so that rates as slow as 30 can be measured readily with camera speeds of 19 to 30 mm. per second.

The caliper is illustrated in the closed position in Fig. 1 and ready for use in Fig. 2. The instrument consists of an adjustable caliper (*B*) and a double rule (*A*), the lower one giving cycle length in hundreds of a second and the upper one heart rate in beats per minute. The scale is 25 cm. long and permits the reading of heart rates between 30 and 400 beats per minute or of cycle lengths between 2.0 and 0.15 seconds. To construct the upper scale, heart rates between 30 and 400 were converted into distance, to be read from the zero point, ranging from $\frac{750}{30}$ cm. = 25 cm. to $\frac{750}{400}$ cm. = 1.875 cm. In the lower scale the subdivisions are 1.25 mm. apart. Each division represents 0.01 seconds. The total length of the scale, 25 cm., therefore contains 200 subdivisions or 2 seconds. The two sections of the rule are separated by a slot (*C*) in which the peg (*E*) can slide.

The caliper is an ordinary enlarging caliper whose longer limbs can be varied in length by the setting of the upper rods (*H* and *H'*, respectively). These rods can be adjusted by loosening the screws (*I*), and once set they are fixed in place by retightening the screws. The shorter limbs (*F*) of the caliper are 2½ cm. long, measured from the fulcrum. The longer limbs (*G*) can be varied from 16 to 25 cm. The rods of the longer limbs are constructed so as to enable them to slide easily in grooves in the fixed part of the longer limbs. One of the rods is fastened to the rule through a pivot that corresponds exactly to the zero point of the scales (*O*). The other rod, as mentioned before, can slide in the slot between the two scales of the rule.

In operating this caliper, the small limbs are applied to the electrocardiographic record so that the points are separated by time lines marking 0.60 of a second. Then the screws (*I*) are loosened and the length of the long limbs

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adjusted so that they bound the distance of 0.60 seconds in the lower scale.* The extra scale on these limbs (*S*) permits setting of both limbs to precisely the same length. The screws (*I*) are then fastened in position, and the caliper is ready for use. The small limbs are applied to mark off the desired distance (R-R or P-P intervals, etc.). The long limbs immediately will give the cycle length and heart rate. When several cycles are measured, the cycle length

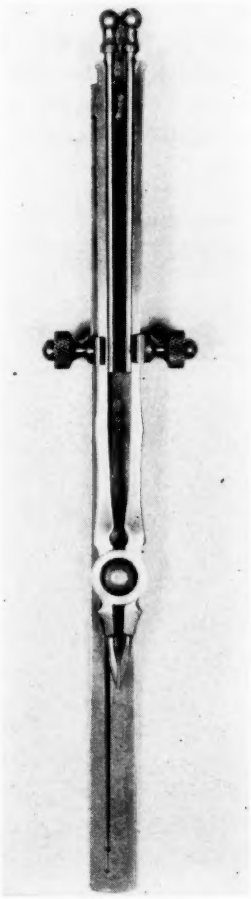

Fig. 1.

can be obtained by dividing the lower scale by the number of cycles measured, and the rate can be obtained by multiplying the reading on the upper scale by the number of cycles measured. In the case of flutter and fibrillation of the auricles, a similar procedure can be used to get the auricular rate.

When used in the manner described, the caliper obviates the inconvenience of fixed rules when camera speed varies. In its construction and use in the

*The rule has an indentation (*D*) in the upper scale set it a cycle length on the lower scale of 0.60 seconds in order to facilitate this operation. A slight upward movement of the rule will remove the peg (*E*) from the notch (*D*) and permit it to slide freely in the slot of the rule.

manner outlined there is, with the usual camera speed, approximately a seven to one enlargement of the scale distance of the record. The enlargement is given by the ratio of the large to small limbs, and it varies from $\frac{16}{2.5}$ to $\frac{25}{2.5}$ or from 6.04 to 10. With a little experience this caliper calculator can be employed quickly. It is especially useful in those institutions which handle a large number of records daily, since the camera speed in a day's run of one machine is sufficiently constant so that once the caliper is set it can be used without readjustment for all of the records of that machine that day. It is also time saving in calculating average rates and cycle lengths in the presence of arrhythmias. However, it must be stressed that its proper use demands a rigorous compliance with the simple rules previously outlined. The caliper has been tested on a large series of records in the Heart Station and found satisfactory in our hands.

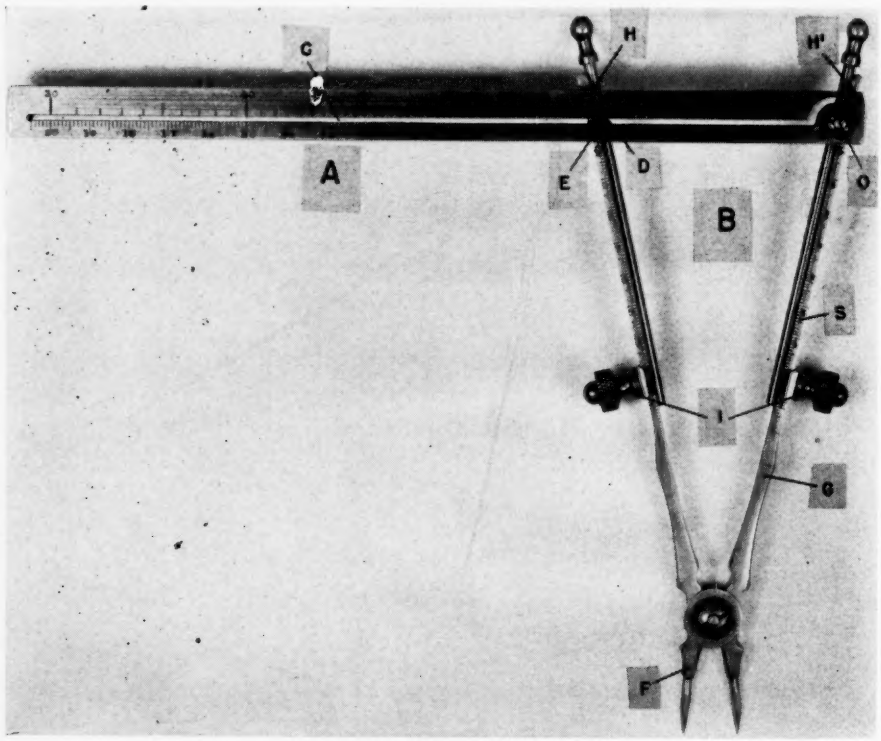

Fig. 2.

SUMMARY

A new caliper calculator for determining heart rate and cycle length in the routine measurement of electrocardiograms is described. The method of its use is outlined, and its advantages for quick calculations are discussed.

We wish to express appreciation to Dr. L. N. Katz, for his guidance in the development of this instrument.

THE DETERMINATION OF URINE UROBILINOGEN

The Interpretation of the Rate of Color Development of the Ehrlich Reaction

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INTRODUCTION

WIDESPREAD recognition of the value of a clinical laboratory test for urobilinogen in the study and diagnosis of liver disease and jaundice has led to the development of a number of methods for the determination of urobilinogen in the urine and stool. The diagnostic value of a purely qualitative test is limited; consequently, efforts have been made to develop a satisfactory quantitative method.

Wallace and Diamond[1] introduced a method involving progressive dilutions of urine. Ehrlich's reagent (paradimethylaminobenzaldehyde in a solution of hydrochloric acid) is added to a series of tubes containing progressive dilutions of urine, and the tube of highest dilution showing evidence of a reddish-pink color is taken as an expression of the quantity of urobilinogen in the urine sample. Five minutes are allowed after the addition of Ehrlich's reagent for production of the "maximum intensity" of color, for it was observed that more color was produced if the reaction was allowed to proceed for some time before readings were taken.

Watson[2-4] has presented a modification of Terwen's[5] extraction method for the quantitative determination of urobilinogen in the urine and stool. This method is the most satisfactory for accurately determining urobilinogen, but it is time consuming and not adaptable to routine clinical use. In recognition of this, Watson[6] recently described a simplified procedure in which color is produced in urine by the addition of Ehrlich's reagent followed by a saturated solution of sodium acetate. The intensity of color is then visually compared with dye standards or measured in the Evelyn photoelectric colorimeter. The solution of sodium acetate both causes maximum development of color and brings the color-producing aldehyde-condensation reaction to a halt. Following the addition of the sodium acetate, the color produced begins to fade gradually, but this is negligible if the colorimeter reading is made within five minutes. Watson points out that in addition to urobilinogen the simplified method also measures other substances in urine which will produce a reddish color when treated with Ehrlich's reagent. Indole and skatole are two of these substances, but there are others about which little is known. Watson, however, states ". . . these substances are usually present in amounts roughly proportional to that of urobilinogen, so that from the clinical standpoint the information gained is usually

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equivalent to that obtained with the exact quantitative method (petroleum ether extraction procedure)."

In the simple direct method as Watson[6] describes it, the immediate addition of sodium acetate to the mixture of urine and Ehrlich's reagent is implied. In some laboratories in which this method is used, it has been customary to modify Watson's procedure by allowing a time interval to elapse between the addition of Ehrlich's reagent and the sodium acetate solution to assure maximum color development. The experiments outlined in the present report suggest that such a modification of Watson's method is not justified and, indeed, that the time interval should be standardized at fifteen seconds.

METHODS AND MATERIALS

Two methods described by Watson (the petroleum ether extraction method[4] and the simplified direct method[6]) were used with the modifications to be described. The material consisted of twelve urine samples from normal individuals and nine urine samples from patients in whom increased amounts of urobilinogen were expected. The latter were all patients with Laënnec's cirrhosis of the liver, with the exception of one patient with infectious hepatitis in early convalescence. All the urine specimens were single voided specimens not accurately representing any constant period of excretion and obtained both in the late morning and in the early afternoon. On all of these urine specimens the simple direct method for the determination of urobilinogen, described by Watson,[6] utilizing the Evelyn photoelectric colorimeter, was used; in addition, the factor of time was studied, readings being made at thirty seconds and five, ten, twenty, and thirty minutes between the time of adding Ehrlich's reagent and the sodium acetate solution. For the sake of standardization, all readings were made thirty seconds after the sodium acetate solution was added and the solutions mixed. In addition, out of the group of nine "abnormal" urine samples five determinations of urobilinogen were carried out by the ether extraction method, described by Watson,[4] utilizing the Evelyn photoelectric colorimeter. This was likewise modified to include the factor of time. After the addition of Ehrlich's reagent to the petroleum ether which contains the extracted urobilinogen and after the extraction of the urobilinogen from the ether by agitation, the Ehrlich fraction was separated and divided into three fractions to which the sodium acetate solution was added at intervals of five, ten, and thirty minutes from the time of adding Ehrlich's reagent to the other extract. In those urines on which both methods were used, the procedures were carried out simultaneously.

RESULTS

The results are shown in Figs. 1, 2, and 3. The intensity of the color which develops in urine before the reaction with Ehrlich's reagent is stopped by the addition of sodium acetate solution (interpreted as Ehrlich units per 100 c.c.), is plotted against the time interval between the addition of Ehrlich's reagent and sodium acetate to the urine. In Fig. 1 the curves are those obtained using the simple method, from the urine of normal individuals; those in Fig. 2

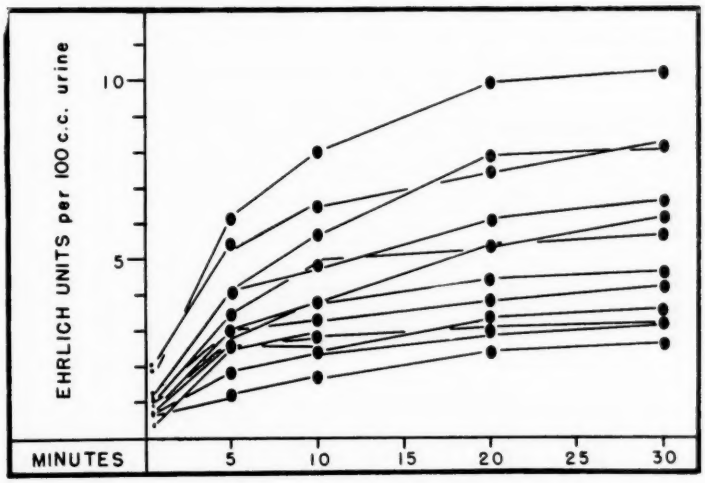

Fig. 1.—Ehrlich reaction in normal urine at various time intervals before addition of sodium acetate.

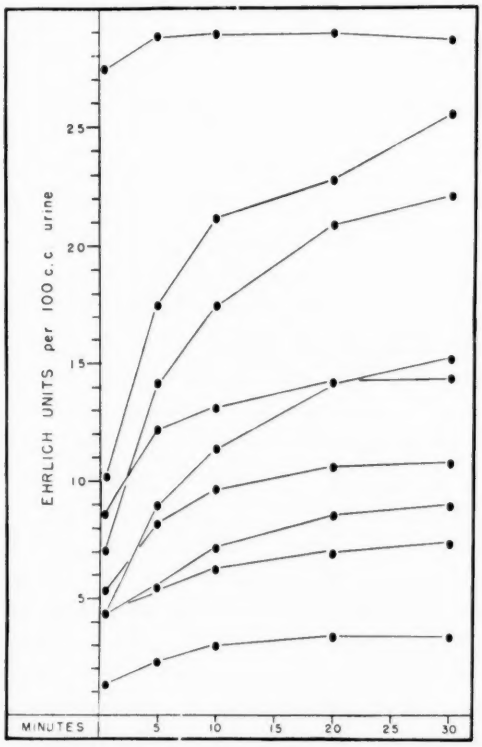

Fig. 2.—Ehrlich reaction in urine from patients with liver disease at various time intervals before addition of sodium acetate.

are from the urines of the patients with liver disease; and those in Fig. 3 are the plotted values obtained when both the simple method and the extraction method were done on four different urine samples.

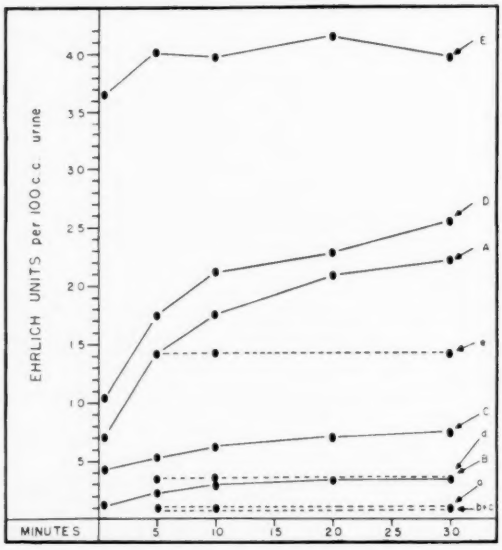

Fig. 3.—Comparison of extraction and simple method for determination of urine urobilinogen.

DISCUSSION

The results indicate that the intensity of color produced in urine after the addition of Ehrlich's reagent will increase with the length of time elapsing between the addition of Ehrlich's reagent and the addition of the sodium acetate solution. This increase in intensity tends to be most rapid in the first ten minutes, thereafter slowing down, and in most cases is negligible at thirty minutes. These data also show that the extracted urobilinogen does not participate in this increase in color intensity. As seen in the curves obtained from both the "normal" urines and the "abnormal" urines, the initial intensity of color bears no constant relation to the maximum intensity developed in thirty minutes. With increasing time between the addition of Ehrlich's reagent and of the sodium acetate solution, a wide variation in the intensity of color produced occurs which makes the distinction between the normal and abnormal urines progressively less marked. Thus, ideally, the sodium acetate should be added immediately after the Ehrlich's reagent; the time at which to read the color produced is as close to zero time as can be done. The values shown for the normals at thirty seconds would indicate that the upper limit of normal expressed in Ehrlich units per 100 c.c. of urine is more than double that suggested by Watson, but if the values are extrapolated back to zero time they would practically all fall within the upper limit of 1.0 Ehrlich unit per 100 c.c. of urine, as Watson indicates. As it is impossible to make readings at zero time,

fifteen seconds has been chosen as the most practical time. Fig. 3 suggests that perhaps the increase in color is not proportional to the amount of urobilinogen present in the urine. Whether this is a major criticism of the method or not cannot be determined from the limited data presented, but it would suggest that further investigation along this line is necessary to establish more accurately the normals for the simple direct method and the relationship if any to the actual amount of urobilinogen present in the urine specimen.

SUMMARY

When Ehrlich's reagent is added to urine, the color produced increases with time. Extracted urobilinogen promptly develops full color with Ehrlich's reagent, which suggests that subsequent increases in color with time are due to substances other than urobilinogen. In liver disease the immediate reaction appears to be the most significant. It is, therefore, recommended that the reaction between Ehrlich's reagent and urine be stopped (by the addition of saturated sodium acetate solution) in as short a time as is convenient and that can be standardized. Exactly fifteen seconds appears to be the most satisfactory time interval.

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BOOK REVIEWS

Everyday Psychiatry. By *John D. Campbell*, M.D., Commander, M.C., U.S.N.R., Chief Neuropsychiatrist, U. S. Naval Base Hospital No. 8; formerly Chief Neuropsychiatrist, U. S. Naval Hospital, Charleston, S. C., and Visiting Lecturer in Psychiatry, Medical College of S. C.; Diplomate, American Board of Neurology and Psychiatry. J. B. Lippincott Company, Philadelphia, 1945. Price $6.00. Cloth with 333 pages.

The author's aim is to present psychiatric medicine to physicians as to make it as naturally acceptable and usable by them as internal medicine, neurologic medicine, gynecologic medicine, or surgical medicine.

To achieve his aim, a simple practical scheme is required for describing and evaluating personality and the common psychiatric disorders. The author presents such a scheme, which involves four "basic personality traits" and two "secondary personality factors." The four basic personality factors are intelligence, conscience, emotional reaction, and psychosexual development. The two secondary personality factors are sociability and special modes of adjustment. Abnormal personalities are ranked (very crudely) on each of these six scales, and when so ranked group themselves into the standard patterns of borderline (nonpsychotic) psychiatric disorder: mental deficiency, psychopathic personality, psychoneurosis and subdivisions, homosexuality, schizoid personality, cycloid personality, chronic alcoholism. These patterns are described in concise, clear, vigorous language, with excellent life histories as illustrations.

In general, the author has succeeded in his task. The physician who uses the book will lose much of his insecurity and anxiety about everyday psychiatric problems. Further, as he faces these problems his interest and skill will increase with resulting benefit to medical practice as well as psychiatry.

There is one regrettable feature of this otherwise very useful book. The author gives the impression (though he denies this as his intention) that the only proper goal of psychiatric treatment is adjusting the patient to his constitutionally given, quantitatively fixed properties or personality factors. This impression ought not to be given for two reasons: (1) Estimation of a patient's properties, by the author's method, is extremely crude, and can be done only within such broad limits as to make meaningless too assured statements about a patient's "constitutional limitations"; (2) experienced psychotherapists see quantitative changes take place in the behavior of patients under treatment; such changes are essential to the success of treatment. It must be said in fairness to the author, however, that no adequate scientific demonstration of their occurrence has yet been made.

GEORGE SASLOW, M.D.

ANNOUNCEMENT

Army Institute of Pathology and American Registry of Pathology

What is now known as the Army Institute of Pathology was established in 1863 as the Army Medical Museum. During World War II the activities of the Institute were greatly expanded, especially in the field of diagnostic pathology and research. There are now on file over 170,000 accessions. The results of research at the Institute during the past few years will be published in a volume of about fourteen hundred pages as a part of the official history of World War II. The present director is Colonel J. Earl Ash, who will be succeeded on October 1 by Colonel Raymond O. Dart.

On request of Major General Norman T. Kirk, Surgeon General of the Army, the Committee on Pathology of the National Research Council, Division of Medical Sciences, in late 1945 prepared a report on the future development of the Institute. The report has been approved by the Surgeon General and by the War Department.

The essential recommendations in this report are: (1) that a new building of adequate size be constructed; (2) that the Institute be organized in four divisions (Department of Pathology, Army Medical Illustration Service, Army Medical Museum, and American Registry of Pathology), each headed by a competent specialist; (3) that the staff of the Institute be drawn from both the commissioned ranks of the Army and from the civilian professions; (4) that a comprehensive educational and training program be undertaken; (5) that the vast store of material at the Institute be used for research; and (6) that the services in pathology in the veteran's hospitals be centralized at the Institute.

The American Registry of Pathology founded in 1922 thus is, and will continue to be, an integral part of the Army Institute of Pathology. There were, on Jan. 1, 1946, over 43,000 cases registered. To effectuate the new plans as they relate to the Registry, the National Research Council, Division of Medical Sciences, appointed a Committee on the American Registry of Pathology. The members of the Committee are Howard T. Karsner, Chairman, Cleveland; Colonel J. E. Ash, Washington; Brigadier General George R. Callender, Washington; Colonel Baldwin Lucke, Philadelphia; Robert A. Moore, St. Louis; Benjamin Rones, Washington; A. R. Shands, Jr., Wilmington; and Henry A. Swanson, Washington.

At the present time there are fourteen registries as a part of the American Registry of Pathology. These include Registry of Ophthalmic Pathology, established in 1922, sponsored by the American Academy of Ophthalmology and Otolaryngology; Lymphatic Tumor Registry, established in 1925, sponsored by the American Association of Pathologists and Bacteriologists; Bladder Tumor Registry, established in 1927, Kidney Tumor Registry, established in 1940, and Prostatic Tumor Registry, established in 1943, sponsored by the American Urological Association; Registry of Dental and Oral Pathology, established in 1933, sponsored by the American Dental Association; Registry of Otolaryngological Pathology, established in 1935, sponsored by the American Academy of Ophthalmology and Otolaryngology; General Tumor Registry, established in 1937, sponsored by the American Society of Clinical Pathologists; Registry of Dermal Pathology, established in 1938, sponsored by the American Academy of Dermatology and Syphilology; Chest Tumor Registry, established in 1942, sponsored by the American Society of Thoracic Surgeons; Registry of Neuropathology, established in 1943, sponsored by the American Association of Neuropathologists; Registry of Orthopedic Pathology, established in 1943, sponsored by the American Academy of Ortho-

pedic Surgeons; Registry of Veterinary Pathology, established in 1944, sponsored by the American Veterinary Medical Association; and Registry of Gerontology, established in 1945, sponsored by the Gerontological Society, Inc.

Plans for additional registries are under consideration. A professional scientific society wishing to sponsor a registry should communicate with the Director, Army Institute of Pathology, 7th Street and Independence Ave, S.W., Washington 25, D. C. The society appoints a committee to work with the Director in supervision of the activities of the Registry and makes an annual contribution to the budget which is administered by the National Academy of Sciences.

All specimens in the Registry are available for review and research by competent investigators. Sets of slides and accompanying syllabuses on special fields are available for loan to the civilian professions and officers in the federal services. Physician, dentists, and veterinarians are urged to send unusual specimens together with an abstract of the history to the Registry. The contributor receives a report on each specimen and is asked to keep the Registry informed of the follow-up on the patient.

With the reorganization of the Army Institute of Pathology to be completed during 1946 and 1947, a full-time scientific director of the American Registry of Pathology will be appointed, and sufficient clerks and technicians will be available to assure adequate use of the registries for diagnosis, research, training of young men, and education of the professions.